THE USE OF PHENYLBORONATE AND STANNATE IN THE SEPARATION AND CHARACTERISATION OF POLYOLS

A thesis submitted by

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Doctor of Philosophy

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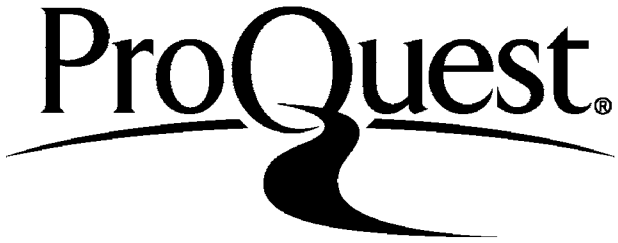

ACKNOWLEDGMENTS

The author would like to acknowledge the help of Professor E. J. Bourne and Dr. H. Weigel, who directed the work.

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CONTENTS

ABSTRACT

The preparation of cyclic phenylboronates by two methods has been described. Phenylboronates from polyols soluble in water were prepared by the method of Kuivila, Keough and Soboczenski.[12] Those from polyols insoluble in water were prepared by the method of Sugihara and Bowman.[2]

A method for estimating the boron content of phenylboronates not containing any other phenyl group has been devised. This utilises the ultraviolet absorption due to the Ø-B< grouping.

The structures of several phenylboronates have been investigated. It has been shown that galactitol forms a 1,3:4,6-bisphenylboronate and that glycerol forms a 1,2-monophenylboronate.

The formation of 5- and 6-membered rings in the phenylboronates of glycerol and galactitol has been discussed with referenceto the reactions of aldehydes and ketones with polyols, and with reference to thermodynamics.

The lack of reactivity of the free hydroxyl groups in some phenylboronates has been discussed and a reason for this suggested. The formation of a 'tridentate' structure, resulting in a boat-shaped conformation in the phenylboronates containing unreactive hydroxyl groups is indicated.

Chromatography of polyols using a solvent containing phenylboronic acid has been carried out. Some correlation between the increase in R_F values of compounds in the presence of phenylboronic acid , and their structures is evident.In most cases it involves a 'tridentate' structure (and 1(ax), 2(eq),3(ax)-grouping of hydroxyl groups) as previously described.

The use of a solution of sodium stannate (at pH 11.5) as an electrolyte for electrophoresis has been described, and it has been shown that for cyclic polyols a 1(ax),2(eq)-diol grouping is preferred, and for acyclic polyols a threo-diol grouping is preferred for complexing.

THE NAMING OF THE COMPOUND WITH THE FORMULA $C_6H_5B(OH)_2$

In scientific literature, three different names are used for this compound. These are phenylboric acid, phenylboronic acid and benzeneboronic acid.

Papers published by Yabroff, Brand and others[1] in the Journal of the American Chemical Society, contain the term phenylboric acid.

The compound has been called benzeneboronic acid by Sugihara and Bowman[2] in J. Amer. Chem. Soc. and more recently by Bourne, Lees and Weigel.[3]

The term phenylboronic acid has been used by, amongst others, Wolfrom and Solms[4] in J. Org. Chem., Ferrier in J. Chem. Soc.[5] and Finch and Lockhart in J. Chem. Soc.[6]

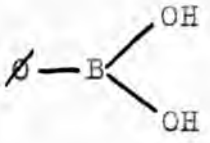
The correct name according to I.U.P.A.C.[7] is benzeneboronic acid. That according to the Ring Index[8] of the American Chemical Society is phenylboronic acid.

In this thesis the term phenylboronic acid is used for the compound with the chemical formula $C_6H_5B(OH)_2$. The phenyl group C_6H_5- is represented by Ø-.

LITERATURE SURVEY

LITERATURE SURVEY

Phenylboronic acid (I) is a white, crystalline solid with a melting point of about 217°C [9].

I 

It can be prepared [10] by the addition of the correct quantity of Grignard reagent to tri-methyl borate (eqn.1), followed by hydrolysis of the ester formed (eqn.2).

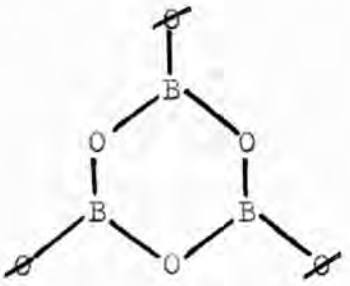
$$\text{ØMgX} + (\text{MeO})_3\text{B} \longrightarrow \text{ØB(OMe)}_2 + \text{MgXOMe} \qquad (1)$$

$$\text{ØB(OMe)}_2 \xrightarrow{\text{hydrolysis}} \text{ØB(OH)}_2 + 2\text{MeOH} \qquad (2)$$

When the acid is exposed to the atmosphere it slowly dehydrates to give the anhydride (II) which has a melting point of about 209-214°C [9].

If the anhydride is dissolved in hot water, and the solution allowed to cool, the acid is precipitated. The acid

$(\phi\text{-BO})_3$



II

can be converted to the anhydride either by heating at 110°C for 5 hours, or by azeotropic distillation using toluene as the solvent [9].

In all cases where accurate quantitative measurements are required, phenylboronic anhydride should be used.

The solubility of phenylboronic anhydride in water is given as 1.7g./100ml. [9] The acid is considerably more soluble. The anhydride is very soluble in ethanol and in diethyl ether and moderately soluble in acetone and benzene [9].

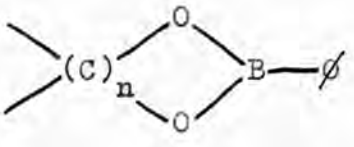
During the 1930's Branch, Yabroff and others [1] published a series of papers relating to phenylboronic acid. The dissociation constants of some 30 substituted phenylboronic acids were determined. The formation of addition complexes of phenylboronic acids and amines was studied, and several crystalline compounds are described.

Diesters of phenylboronic acid [11] are readily prepared

by heating the anhydride with a monohydric alcohol, in the presence of an entraining agent, and removing the water formed as an azeotropic mixture (eqn.3).

$$\phi_3B_3O_3 + 6ROH \longrightarrow 3\phi B(OR)_2 + 3H_2O \qquad (3)$$

The preparation of cyclic phenylboronate esters has also been described. These compounds contain heterocyclic rings of different sizes which contain carbon, oxygen and boron atoms.

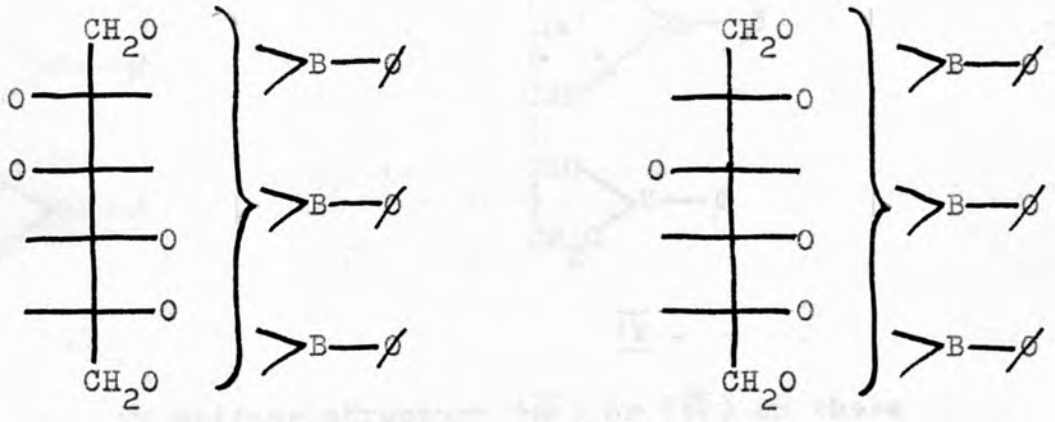


'n' is an integer

Several different methods have been described for the preparation of these cyclic esters.

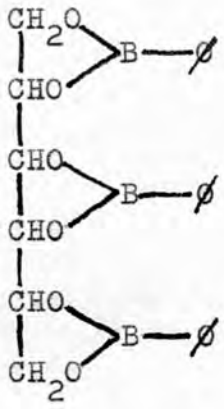
In 1954 Kuivila, Keough and Soboczenski [12] described the preparation of certain arylboronates of some diols and polyols. The method they employed consisted of mixing a solution of the diol or polyol in water, with a solution of the arylboronic acid in methanol. Amongst the arylboronates reported were the trisphenylboronates of D-mannitol (A) and

D-glucitol (B).

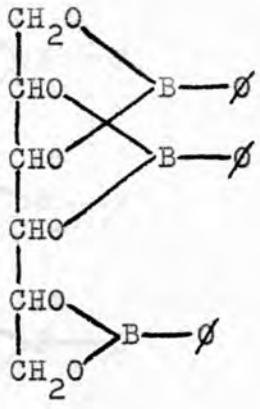


A B

These authors gave no evidence as to the detailed structures of the arylboronates prepared, although certain suggestions were made about the most likely ring sizes of the cyclic esters of the polyols. They suggest that the most likely structure for D-mannitol trisphenylboronate is that containing three five-membered cyclic phenylboronate esters (III). They also suggest a structure (IV) containing two six-membered rings and one five-membered ring, and say that this structure appears to be possible on stereochemical grounds.



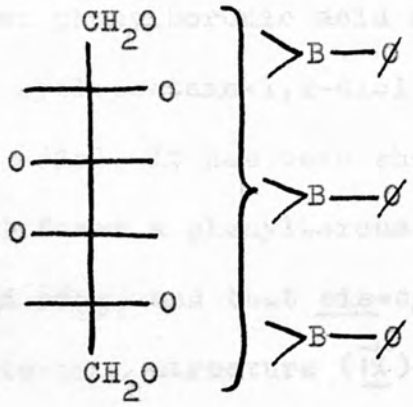
III



IV

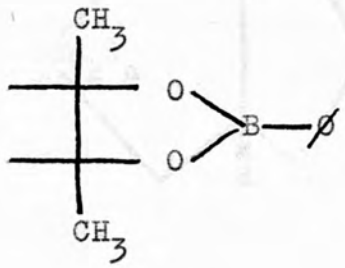
However, in neither structure (III) or (IV) do these authors consider the relationships between the hydroxyl groups in D-mannitol, and make no attempt to relate the reaction of the phenylboronic acid with the structure of D-mannitol when the carbon atoms form a planar zig-zag chain. In the formation of structure (IV) from D-mannitol, the action of phenylboronic acid cannot be compared with the reaction of either aldehydes or ketones with D-mannitol.

Certain cyclic phenylboronate esters were prepared by Sugihara and Bowman (1958)[2] by refluxing the components in anhydrous or aqueous acetone. They reported the preparation of the trisphenylboronates of D-mannitol (A), D-glucitol (B), and galactitol (C), but gave no information about the detailed structures of these compounds.

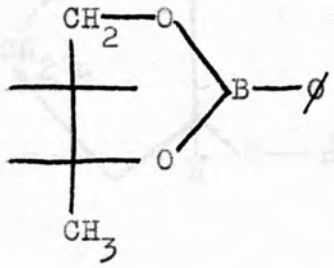


C

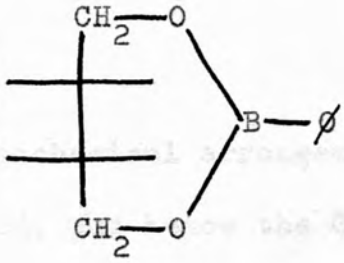
They also prepared cyclic phenylboronates from butane-2,3-diol (V), butane-1,3-diol (VI) and butane-1,4-diol (VII), i.e. five-, six- and seven-membered rings.



V

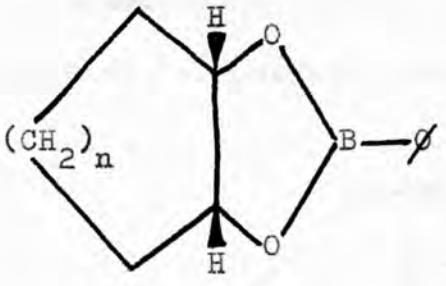


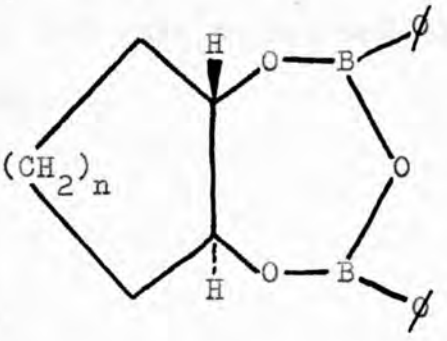
VI



VII

It was found that phenylboronic acid reacted with both cis- and trans- cyclopentane-1,2-diol and cis- and trans-cyclohexane-1,2-diol. It has been shown that the cis-cyclopentane-1,2-diol forms a phenylboronate with structure (VIII), a five-membered ring, and that cis-cyclohexane-1,2-diol forms a phenylboronate with structure (IX), again a five-membered ring. Elemental analysis and molecular weight determinations indicated that the trans-diol arrangements in the cyclopentane- and cyclohexane-1,2-diols formed seven-membered pyrophenylboronate structures (X) and (XI).





VIII n = 1
IX n = 2

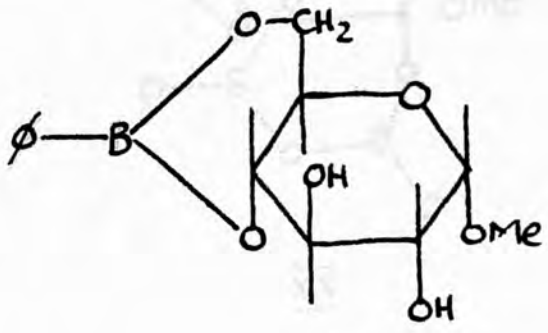
X n = 1
XI n = 2

Thus the stereochemical arrangement of the hydroxyl groups of a compound, and hence the O-O distances in the diol groups concerned are responsible, together with the

stereochemistry of the phenylboronic acid, for the type of compound produced.

Sugihara and Bowman[2] obtained an amorphous solid from the reaction of methyl β-D-glucopyranoside and two mol. of phenylboronic acid, but its detailed structure was not determined.

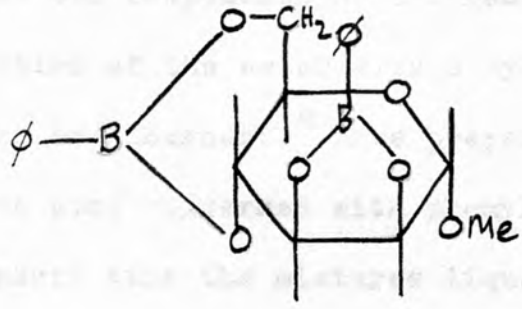
Ferrier[5] has examined the interaction of phenylboronic acid (and anhydride) with hexosides. Phenylboronates were prepared by azeotropic distillation using benzene or dioxan as the solvent. It was found that, depending on the proportions of phenylboronic acid to hexoside, different products could be obtained. Molar proportions of 1:1 gave methyl α-D-gluco-pyranoside-4,6-phenylboronate (XII).



XII

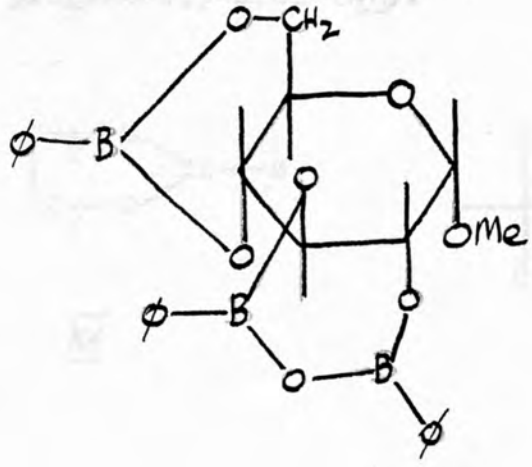
Molar proportions of 2:1 gave methyl α-D-mannopyranoside-2,3:4,6-phenylboronate (XIII), but 2:1 proportions with

methyl α-D-glucopyranoside gave a non-crystalline mixture.



XIII

Molar proportions of 3:1 gave methyl α-D-glucopyranoside-2,3(diphenylpyroboronate)4,6-phenylboronate (XIV).

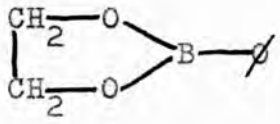


XIV

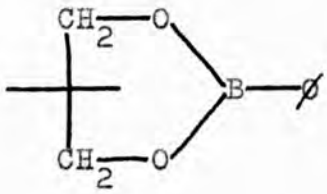
Wolfrom and Solms[4] have prepared crystalline phenylboronates from the pentoses and several 6-deoxy-hexoses, but no indication of the detailed structures of these compounds

has been given. Their method of preparation consisted of fusing the two components of the reaction together, followed by extraction of the ester with a hydrocarbon solvent.

Finch and Lockhart [6] have prepared phenylboronates by mixing the diol concerned with phenylboronic anhydride. After a short time the mixtures liquefied and the cyclic compound and water were formed. The water was mechanically separated from the phenylboronate which was distilled, after drying by shaking with magnesium sulphate. Compounds prepared by this method include the phenylboronates of ethylene glycol (XV) and propane-1,3-diol (XVI).



XV



XVI

There was no reaction on mixing phenylboronic anhydride and butane-1,4-diol, although the phenylboronate of this diol has been reported [2] (VII p.14).

It has been shown that a variety of diol systems will react with phenylboronic acid to form five-, six- or seven-

membered cyclic phenylboronate esters, when the O-O distances in the diols concerned are suitable compared with the stereochemistry of phenylboronic acid.

INTRODUCTION

INTRODUCTION

Consideration of the information available, concerning the reaction between phenylboronic acid and polyols indicates certain possible uses of this reaction and certain aspects which deserve further investigation.

Initially, there is a possibility of using phenylboronates as derivatives for the characterisation of polyols. The phenylboronates of polyols so far reported are crystalline solids with definite melting points. In many cases they can be prepared from aqueous solutions of the polyols. This could be advantageous in a reaction where a polyol is produced by hydrolysis in an aqueous solution, as the preparation of a phenylboronate would not require the isolation of an anhydrous specimen of the polyol.

It has been shown that five-, six- and seven-membered cyclic phenylboronate esters are possible. Information about the type of diol grouping preferred for reaction with phenylboronic acid, and whether phenylboronic acid in its reaction with diols, shows any resemblance to the reactions of either aldehydes or ketones with diols, can be obtained by several methods.

The reaction of partially substituted polyols with phenylboronic acid will give additional information about diol groupings which react with phenylboronic acid.

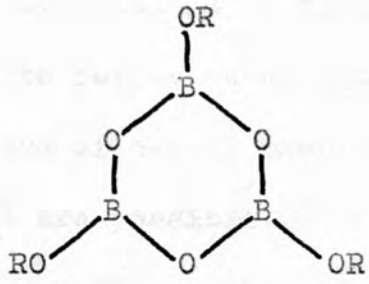
The study of fully substituted phenylboronates such as the tris-esters of D-mannitol and D-glucitol (A&B), will give an indication of the diol groups preferred for reaction, and in addition, information about the preferred stereochemical requirements for reaction. Determination of the detailed structures of the phenylboronates of hexitols will enable comparisons to be made with the reactions of al aldehydes and ketones with polyols. In studying a variety of such reactions Barker and Bourne[13] have compiled certain rules, concerning the preferred hydroxyl group arrangement in a polyol for reaction with an aldehyde or ketone. When aldehydes react with polyols, the first preference is for a βC[14] arrangement., and secondly for a β arrangement. Thirdly the preference is for an α, αT, βT or γT ring, but in benzylidenation and ethylidenation, an αT arrangement takes precedence over a βT or γT arrangement. (The nomenclature used for describing diol arrangements in acyclic polyols is that explained by Barker and Bourne[14]).

It is possible that a study of the infrared absorption

spectra of phenylboronates may be of interest in determining the sizes of the phenylboronate rings in the molecule. The infrared spectra of boron compounds have been studied in some detail and certain frequency assignments have been made for e.g. B—O, B—N and B—C bonds.[15] However most of the compounds studied have been acyclic ones, and there is little information about the spectra of cyclic organo-boron compounds.

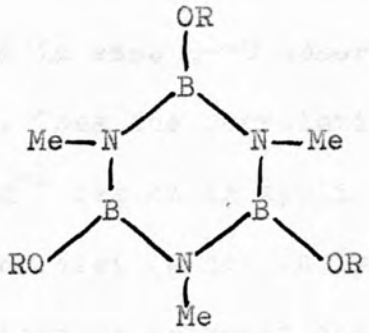
Bellamy[15] et.al. have studied the infrared spectra of a number of B-aryl compounds. They have noted the presence of a strong band between 1440 and 1430cm^{-1} which they have attributed to the same source as the 1470-1438cm^{-1} band occurring in the spectra of most mono-substituted aromatic compounds. The spectra of these B-aryl compounds also show strong absorption between 1280 and 1250cm^{-1} which has been assigned to the B—Ø bond. In the spectra of di-alkyl phenylboronates this band is lower and is seen at 1175-1125cm^{-1}. Certain bands in the infrared spectra of boron compounds can be attributed to the B—O assymetric stretching vibration. The position of these bands in the spectra varies, and depends on the type of boron compound concerned.

Lappert[16] has studied the spectra of several alkyl metaborates with structures (XVII), where R = an alkyl group.



XVII

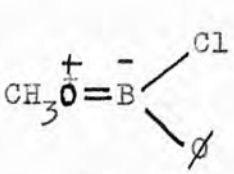
He states that bands at 1486cm^{-1} are associated with the B—O assymetric stretching vibration. Bradley et.al.[17] have found that in the spectra of substances of type (XVIII), where R = an alkyl group, bands at 1330-1318cm^{-1} are caused by the B—O stretching vibration.



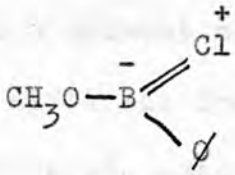
XVIII

In the spectra of the B-aryl compounds studied by Bellamy et. al.[15] it was found that the B—O stretching

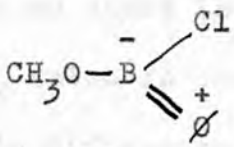
absorption was at 1350-1310cm^{-1}. This absorption was thought to be due to resonance within the molecule; for example, in the case of methyl phenylchloroboronate structures (XIX), (XX) and (XXI) are possible.



XIX



XX



XXI

It is found that the strong band at about 1340cm^{-1} is absent when boron compounds are coupled with strong electron donors, e.g. pyridine or tertiary bases, as this coupling suppresses the resonance effect. It becomes difficult to recognise the less intense B—O absorption occurring at lower frequencies. Thus the correlation of the strong peak in the 1350-1310cm^{-1} region is applicable only to structures in which the boron octet is not fully completed, i.e. only to structures containing trigonal boron.

There have been no particular frequencies described which can be said to be characteristic of five- or six-membered phenylboronate rings, but it is possible that if the spectra of enough phenylboronates were available, that some correlation

between infrared absorption and ring size could be made.

Another method of studying the preferred diol grouping for reaction with phenylboronic acid, is by the incorporation of phenylboronic acid into a chromatography solvent. Boric acid has been incorporated into such solvents.[18] The R_F value of a compound in a solvent containing an added inorganic material is sometimes different from the R_F value in the solvent without the inorganic material. This difference in R_F values can be related to the reaction between the compound and the inorganic material. Thus it is possible that a study of the R_F values of polyols on chromatography using solvents with and without phenylboronic acid can be used to determine the diol groupings most favourable for reaction with phenylboronic acid.

PREPARATION OF PHENYLBORONATES AND ANALYTICAL METHODS

THE PREPARATION OF PHENYLBORONATES AND ANALYTICAL METHODS

A number of cyclic phenylboronate esters have been prepared. Two methods were used. Phenylboronates of water-soluble polyols were prepared by precipitation from aqueous methanol[12], and those of substituted polyols, insoluble in water, by refluxing the components in acetone[2]. In addition, a sample of methyl α-D-glucopyranoside-4,6-phenylboronate was prepared by azeotropic distillation in benzene solution, according to Ferrier.[5]

Analytical Methods

1. Analysis for Boron (Expt. 38)

The estimation of boron in an organo-boron compound, when the boron atom is attached to a carbon atom requires drastic treatment to break this B—C bond. A method has been devised by which the boron content of a phenylboronate can be determined without the necessity of breaking this bond. This method depends on the ultraviolet absorption spectrum of the ø—B< group.

The ultraviolet absorption spectrum of phenylboronic

acid in a solution of methanol/water (50% v/v) was plotted using a Unicam (SP 500) instrument. At suitably low concentrations, the spectrum consists of a single peak at 219 mμ Fig.1.

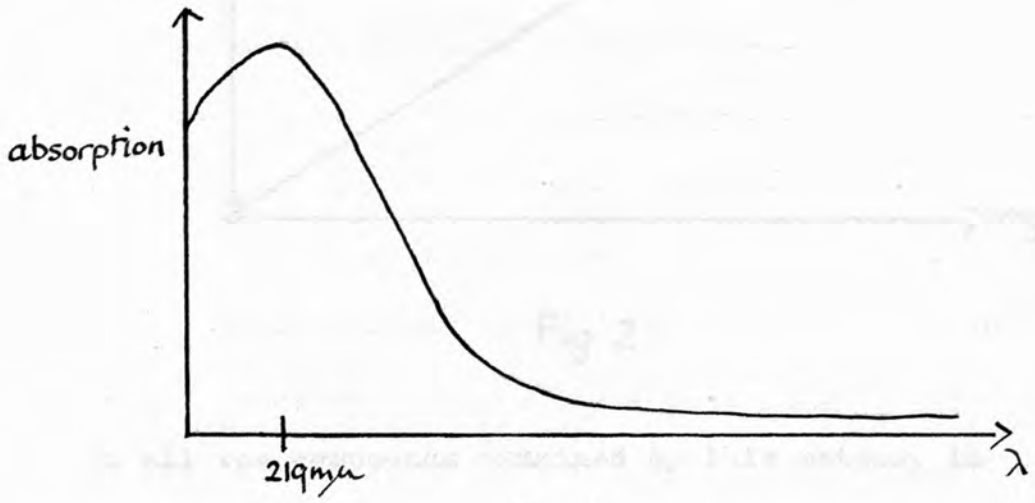


Fig. 1.

A standard solution of phenylboronic acid in aqueous methanol (50% v/v) was prepared, and diluted to a number of known concentrations. The absorption at 219 mμ at each of these concentrations was determined, and the results plotted on a graph. Fig.2.

From this graph it was possible to determine the quantity of boron (in the form of ø—B<) in a solution of a phenylboronate in aqueous methanol.

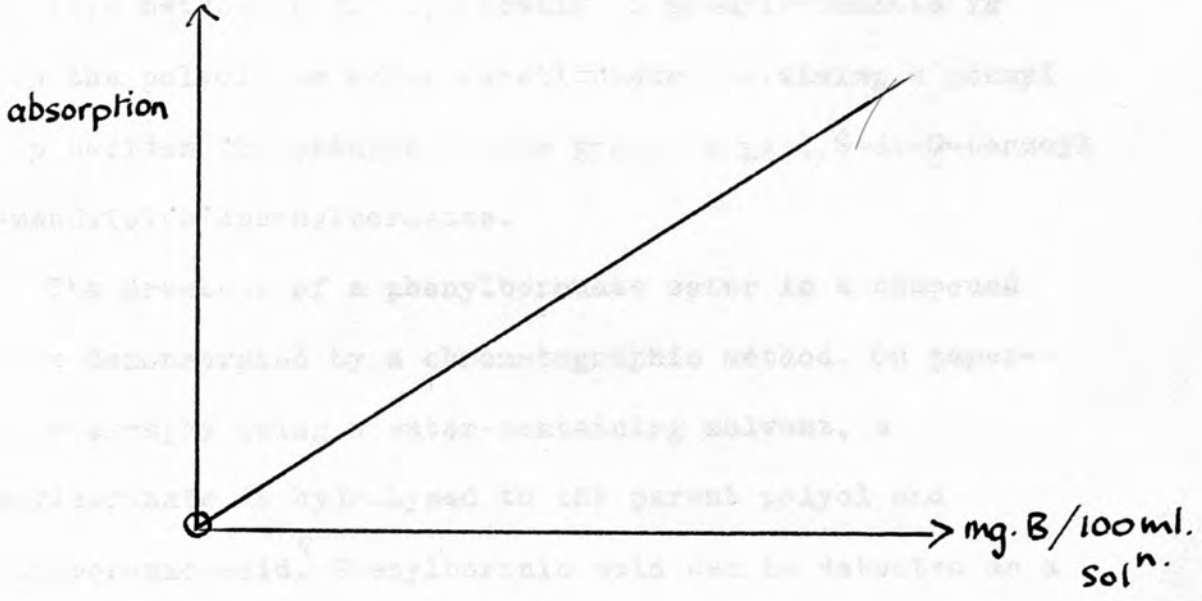
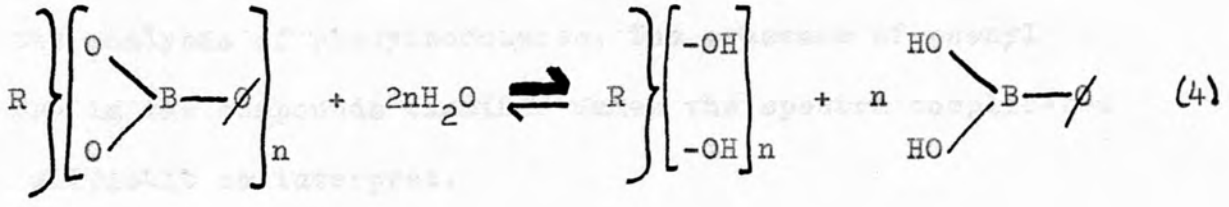


Fig. 2.

In all the compounds examined by this method, in aqueous methanol, and at very low concentrations, it is most likely that the phenylboronate has been hydrolysed to the polyol and phenylboronic acid.



(4)

However this hydrolysis is not essential to the method. The absorption is dependant on the Ø—B< grouping.

This method is not applicable to phenylboronates in which the polyol has other substituents containing a phenyl group besides the phenylboronate group, e.g. 1,6-di-O-benzoyl -D-mannitol-bisphenylboronate.

The presence of a phenylboronate ester in a compound can be demonstrated by a chromatographic method. On paper-chromatography using a water-containing solvent, a phenylboronate is hydrolysed to the parent polyol and phenylboronic acid.[4] Phenylboronic acid can be detected as a grey spot, by the reagent silver nitrate/sodium hydroxide solution[19], and has an R_F value slightly less than 1. In addition, it is visible under ultraviolet light as a purple spot.

2. Infrared Absorption Spectra (Expt 40)

Infrared absorption spectra have been used qualitatively in the analysis of phenylboronates. The presence of phenyl groups in the compounds examined makes the spectra complicated and difficult to interpret.

However there are two absorption peaks in the spectra which give some information about the structures of phenylboronates.

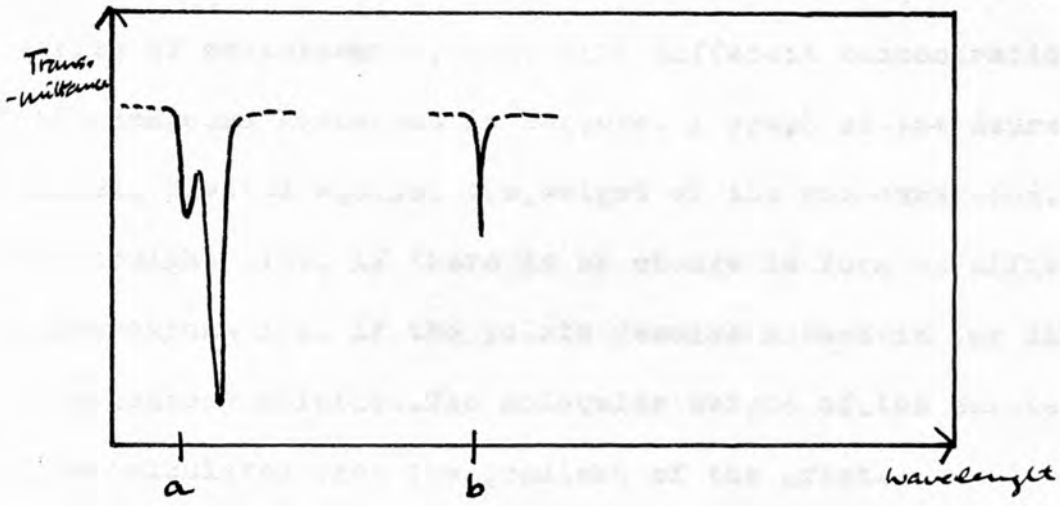


Fig.3.

The peak 'a', in Fig.3., at about 3400cm^{-1} is present in the spectra of all compounds containing free hydroxyl groups. It is therefore present in the spectra of polyols. The absence of a peak 'a' in the spectrum of a phenylboronate indicates that there are no free hydroxyl groups in the molecule. The peak 'b' at 1600cm^{-1} is diagnostic for an aromatic ring and therefore its presence in the spectrum of a compound resulting from a reaction between a polyol and phenylboronic acid, confirms the presence of a phenylboronate ester.

3.Molecular Weight Determination (Expt 36)

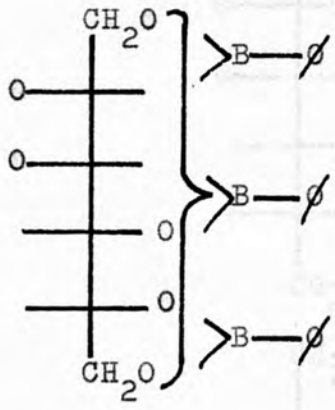
The molecular weights of compounds soluble in benzene can

be determined by cryoscopy. Accurate determinations require a series of measurements, made with different concentrations of the compound concerned in benzene. A graph of the depression obtained, plotted against the weight of the compound used, is a straight line, if there is no change in form at different concentrations i.e. if the solute remains monomeric (or dimeric) in the benzene solution.The molecular weight of the solute can be calculated from the gradient of the graph.

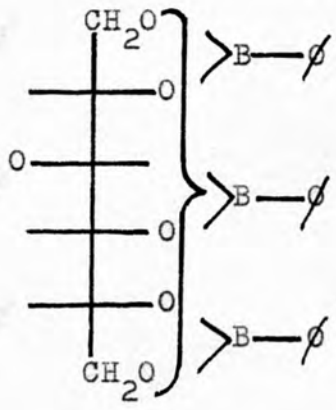
Preparation of Phenylboronates

1.From D-mannitol and D-glucitol

When solutions of D-mannitol and D-glucitol in water and solutions of phenylboronic acid in methanol (in 1:3 molar ratio) were mixed, white precipitates were obtained. (Expts. 1 and 2). In each case, analysis gave 62.92C, 4.92H, 7.4%B, which is correct for D-mannitol and D-glucitol trisphenylboronates (A and B).



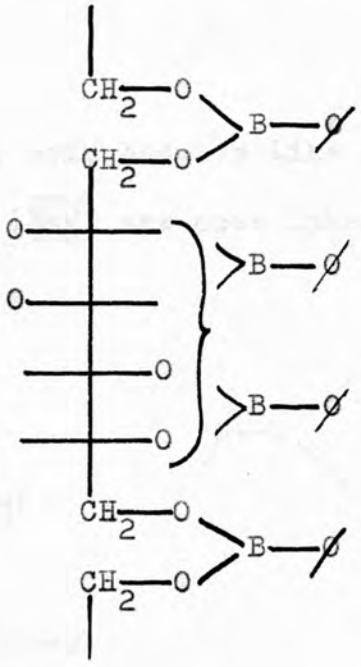
A m.p.137°C



B m.p.189°C

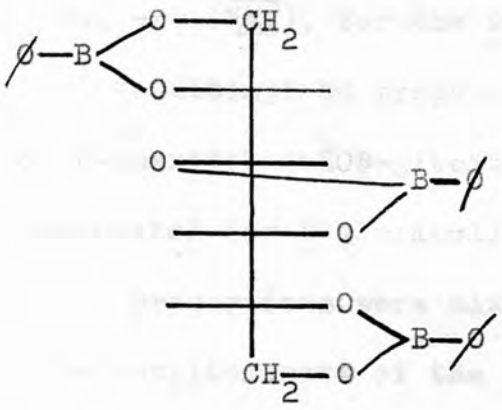
The infrared spectra of A and B had no peak at 3400cm^{-1} indicating that in both compounds the polyol is fully substituted. Both spectra contained a peak at 1600cm^{-1} confirming the presence of phenylboronate ester groups.

Thus it is likely that the phenylboronates of D-mannitol and D-glucitol have structures A and B. An additional possibility is that of a polymeric structure, e.g. D, but in the case of the D-mannitol ester, this was disproved, because the molecular weight was found to be 440 (Theoretical for $C_{24}H_{23}O_6B_3$ 440). (Expt. 36)

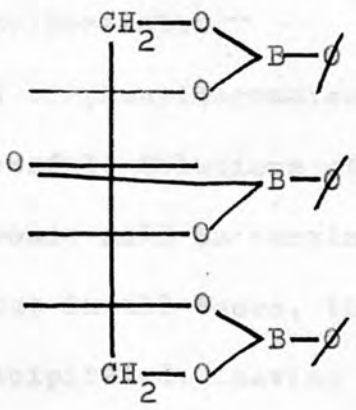


D

There are several possible structures for the compounds A and B. If the reaction of phenylboronic acid is comparable to that of a ketone, then structures (XXII) and (XXIII) are possible.

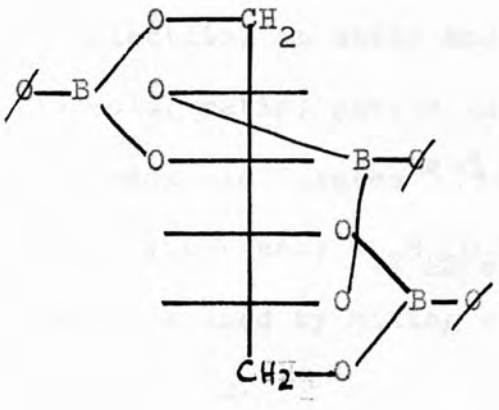


XXII

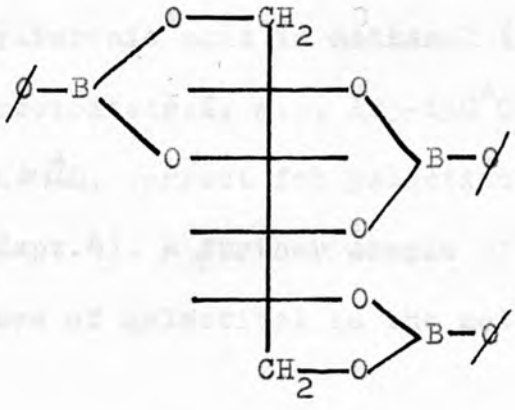


XXIII

If phenylboronic acid behaves like an aldehyde then structures (XXIV) and (XXV) are more likely.



XXIV



XXV

Structure (XXIV) for compound A is seen to be different from structure (IV)[12] for A. Structure (XXIV) contains two six- and one seven-membered ring, whereas structure (IV) containing two six- and one five-membered ring is comparable with

structure (XXV), for the D-glucitol phenylboronate.

An attempt to prepare the mono- and bisphenylboronates of D-mannitol and D-glucitol was unsuccessful. Solutions of D-mannitol (or D-glucitol) and phenylboronic acid in varying molar proportions were mixed together, but in all cases, the trisphenylboronate of the polyol was precipitated, leaving in solution the excess polyol. (Expt. 3.)

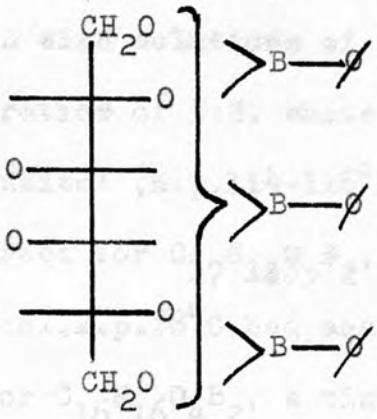
2.From Galactitol

In contrast to the method of Sugihara and Bowman [2] which gave a galactitol tris phenylboronate, C, a mixture of solutions of galactitol in water and phenylboronic acid in methanol (in 1:3 molar ratio) gave a white precipitate,E, m.p. 125-130°C. with analysis figures 59.9% C 6.0% H 6.3% B, correct for galactitol bisphenylboronate $C_{18}H_{20}O_6B_2$. (Expt.4). A further sample of E was prepared by mixing solutions of galactitol in the molar ratio of 1:2.

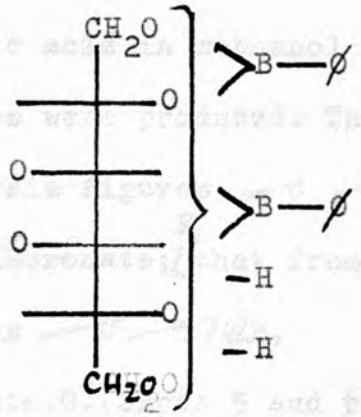
There are several possible structures for the substance E including (XXVI) which would be expected if phenylboronic acid acts in a similar way to an aldehyde, and (XXVII).

The infrared spectrum of E showed a peak at 3400cm^{-1}, of reduced intensity compared with the same peak in the spectrum

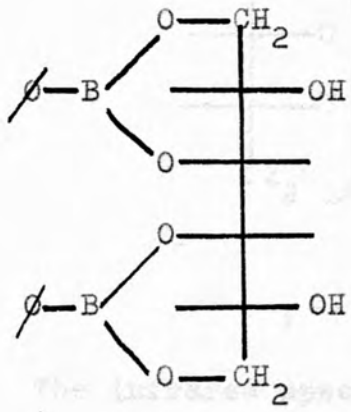
of galactitol, and thus indicating a reduction in the number of hydroxyl groups in the molecule. A peak at 1600cm^{-1} confirmed the presence of phenylboronate ester groups.



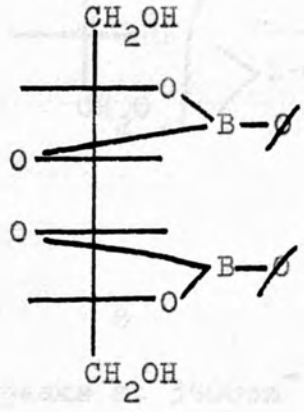
C



E



XXVI



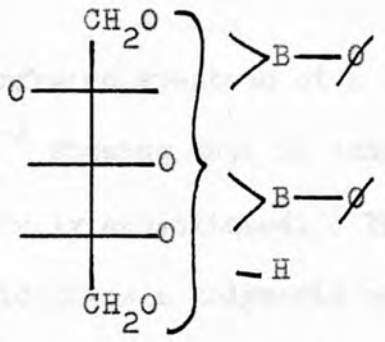
XXVII

The insolubility of E in benzene prevented the determination

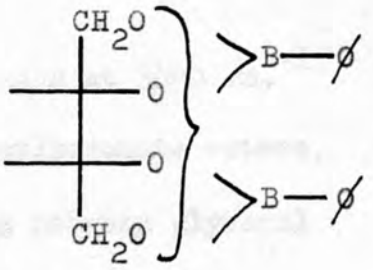
of the molecular weight of the compound.

3.From D-arabitol and D-erythritol

When solutions of D-arabitol and D-erythritol in water were mixed with solutions of phenylboronic acid in methanol in molar ratios of 1:2, white precipitates were produced. That from D-arabitol ,m.p.114-116°C, had analysis figures ~~C~~ ~~H~~ 6.7%B, correct for $C_{17}H_{18}O_5B_2$, a bisphenylboronate; F, that from D-erythritol,m.p.88°C had analysis figures ~~C~~ ~~H~~ 7.4%B, correct for $C_{16}H_{16}O_4B_2$, a bisphenylboronate,G.(Expts 5 and 6).



F

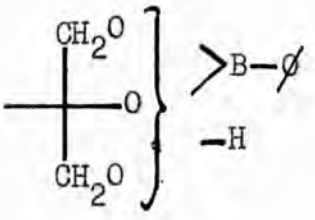


G

The infrared spectrum of F included peaks at 3400cm^{-1} (hydroxyl group) and 1600cm^{-1}, whereas that of G had no absorption at 3400cm^{-1}, but included a peak at 1600cm^{-1}.

4. From glycerol

When solutions of glycerol in water and phenylboronic acid in methanol were mixed together there was no precipitate. When a large excess of glycerol was added to the mixture, a white precipitate was formed, with m.p. 74.5-76.5°C, and analysis figures, 60.0%C, 6.1%H, 6.0%B, correct for $C_9H_{11}O_3B_1$, a glycerol monophenylboronate, H. (Expt. 7).

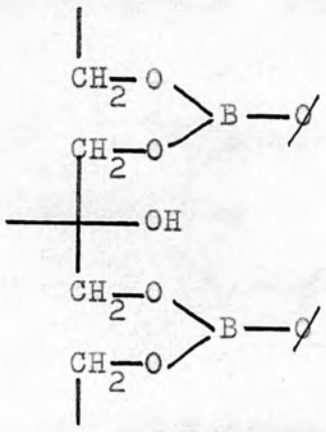


H

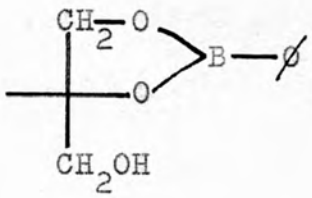
The infrared spectrum of H included peaks at 3400 $cm.^{-1}$ and 1600 $cm.^{-1}$ showing that it contained phenylboronate esters, but was not fully substituted. The reaction between glycerol and boric acid gives a polymeric compound with formula $(C_3H_5BO_3)_x$[20], and the possibility of the production of a polymer e.g. (XXVIII) from the reaction between glycerol and phenylboronic acid could not be discounted.

However, the molecular weight of H was found to be 178 (theoretical for monomer, 178), and thus H must have structure

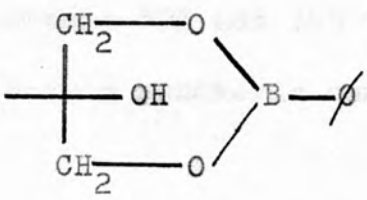
(XXIX) or (XXX).(Expt.36)



XXVIII



XXIX

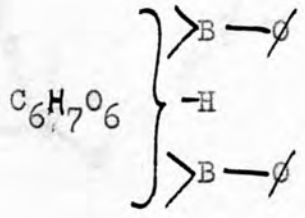


XXX

5.From D-glucose

When solutions of D-glucose in water and phenylboronic acid in methanol were mixed, there was no precipitate. However if the mixture was allowed to stand overnight, an oil separated, below the aqueous methanol mixture. This eventually solidified, giving a white solid,m.p.166°C, with analysis figures,61.2%C; 5.3%H, 6.1 %B, correct for $C_{18}H_{18}O_6B_2$, a glucose bisphenylboronate, I.(Expt.8)

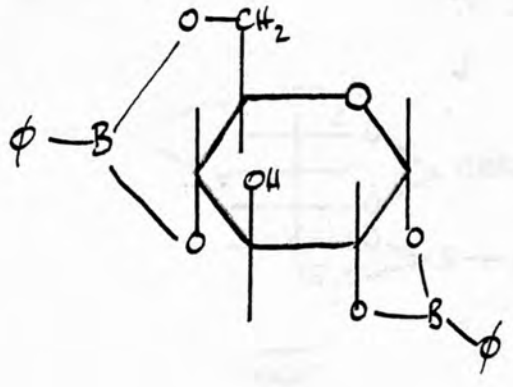
The infrared spectrum of I included peaks at $3400cm^{-1}$ (hydroxyl group) and at $1600cm^{-1}$ (phenylboronate ester group).



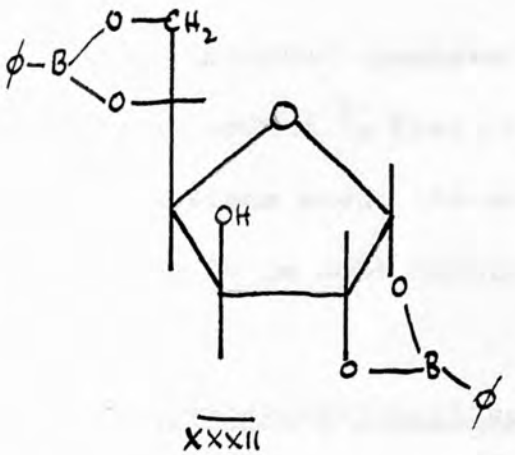
I

A molecular weight determination was carried out on this compound with difficulty, because of its limited solubility in benzene; however, the value of between 325 and 365 (theor. for monomer, 352) indicates that it is a monomeric compound. (Expt.36)

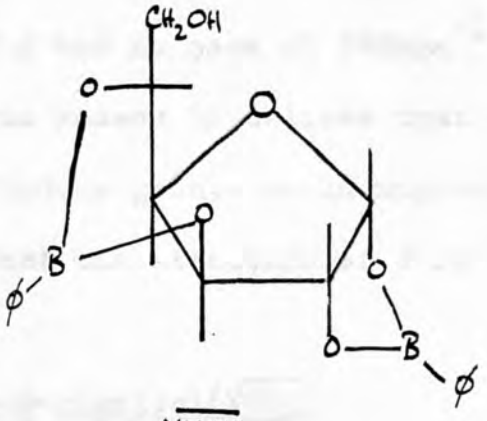
There is a number of possibilities for the structure of compound I. The glucose part may be in the pyranose or furanose form, and for each form different phenylboronate rings are possible, e.g. XXXI - XXXII.



XXXI



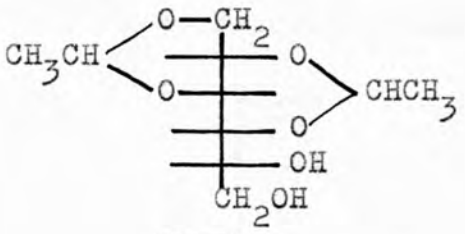
XXXII



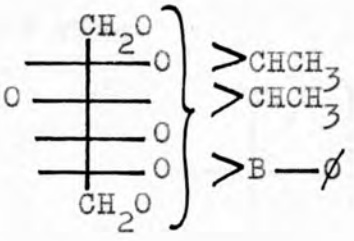
XXXIII

6.From 1,3:2,4-di-O-ethylidene-D-glucitol(XXXIV)

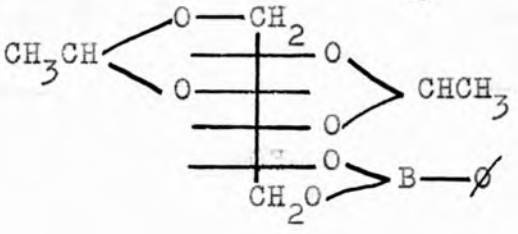
When the compound (XXXIV) and phenylboronic acid were refluxed together in acetone, and the acetone then removed, a white solid,J, was produced, with m.p. 88°C and analysis figures 59.6% C, 6.1% H, 3.3% B, correct for $C_{16}H_{21}O_6B$, a monophenylboronate. (Expt.9)



XXXIV



J



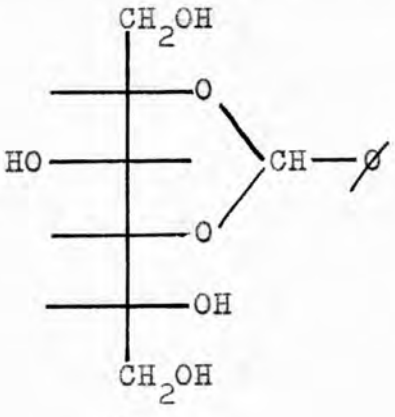
XXXV

The infrared spectrum of J had no peak at 3400cm^{-1} and a peak at 1600cm^{-1}. There is no reason to believe that under the conditions used, the ethylidene groups would migrate, and therefore it is most likely that the structure of J is (XXXV).

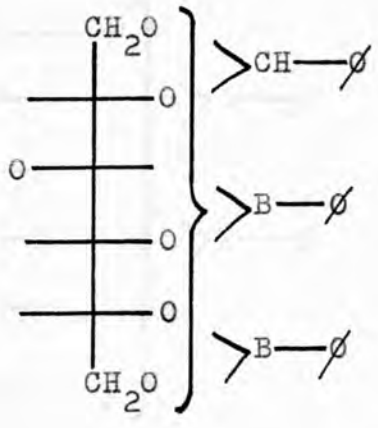
7.From 2,4-mono-O-benzylidene-D-glucitol(XXXVI)

When the compound (XXXVI) and phenylboronic acid (1:2 molar ratio) were refluxed together in acetone,and the acetone removed, a white solid K was produced, with m.p.199°C and analysis figures 67.9% C, 5.0% H, correct for $C_{25}H_{24}O_6B_2$, a bisphenylboronate. (Expt.10.)

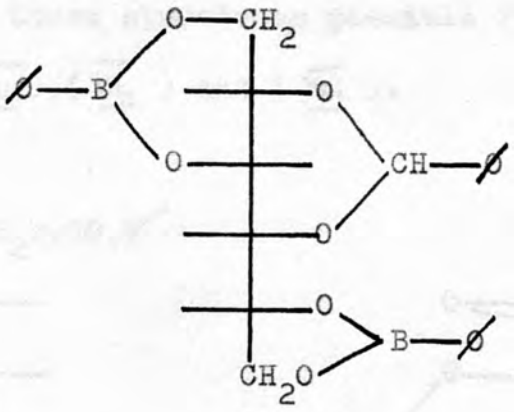
The infrared spectrum of K had no peak at 3400cm^{-1}, indicating an absence of free hydroxyl groups. It is likely that the structure of K is (XXXVII), containing one five- and one six-membered phenylboronate ring.



XXXVI



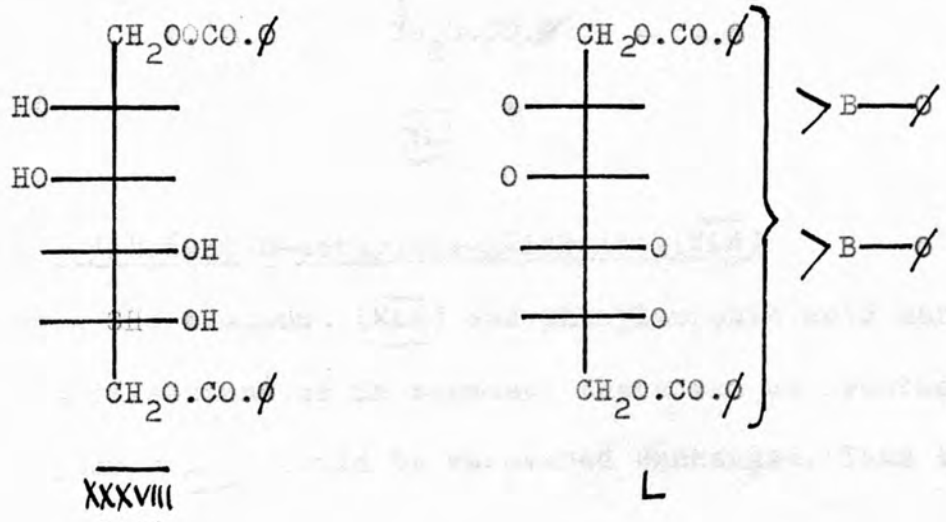
K



XXXVII

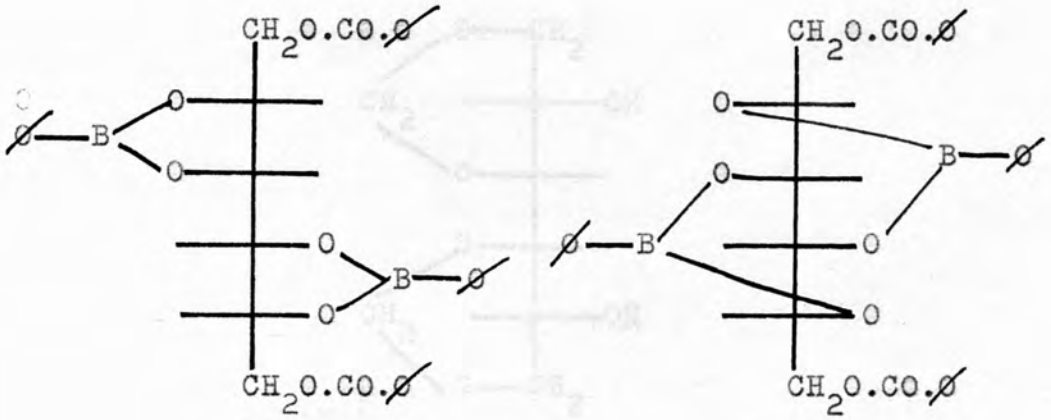
8. From 1,6-di-O-benzoyl-D-mannitol (XXXVIII)

When the compound (XXXVIII) and phenylboronic acid (1:2 molar ratio) were refluxed together in acetone, and the acetone removed, a white solid L was produced, m.p. 150°C, and analysis figures 67.9%C, 4.9%H, correct for $C_{32}H_{28}O_8B_2$, a bisphenylboronate, (Expt.11.) The infrared spectrum of L had no absorption at 3400cm^{-1}.



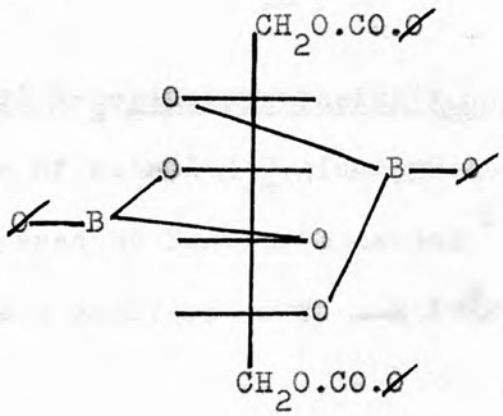
XXXVIII L

There are three structures possible for the compound L, structures (XXXIX), (XL) and (XLI).



XXXIX

XL

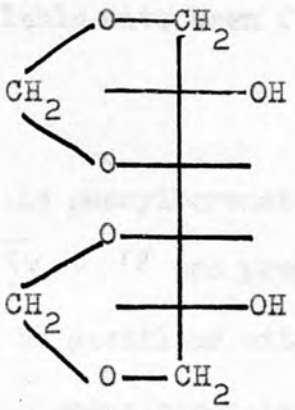


XLI

9.From 1,3:4,6-di-O-methylene-galactitol(XLII)

When the compound (XLII) and phenylboronic acid were refluxed in acetone or in benzene, there was no reaction and the compound (XLII) could be recovered unchanged. Thus the

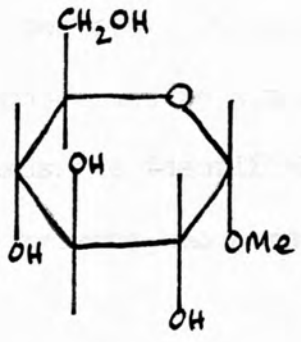
seven-membered ring, with the hydroxyl groups in the γC arrangement cannot be formed.(Expt.12)



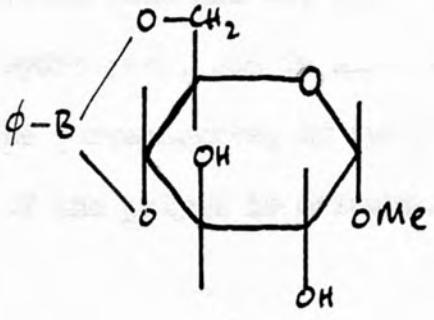
XLII

10.From methyl α-D-glucopyranoside(XLIII)

A sample of methyl α-D-glucopyranoside-4,6-phenylboronate (XII) was prepared by Ferrier's method [5], and found to have m.p.166.5°C and analysis ~~C H~~ 3.8%B, correct for $C_{13}H_{17}O_6B$. (Expt.13)



XLIII



XII

The stability of the phenylboronates of polyols

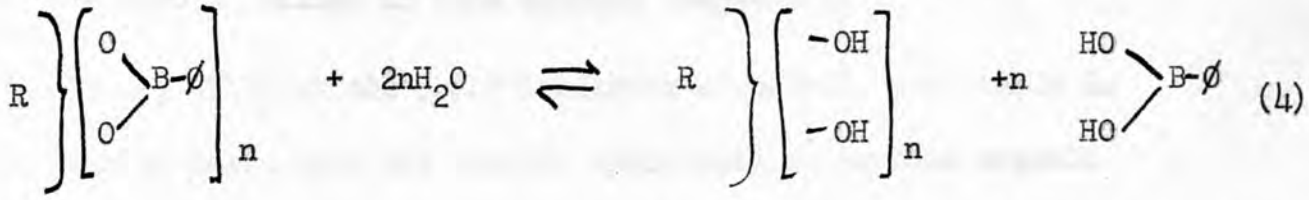
Coates[21] states that cyclic boronic acid esters are somewhat more stable than the non-cyclic esters. However, the cyclic phenylboronates available have been found to be very unstable under certain conditions.

The liquid cyclic phenylboronates (e.g. ethylene glycol monophenylboronate XV p. 18 and propane-1,3-diol-monophenylboronate XVI p.18) can be distilled without decomposition. The available solid cyclic phenylboronates are crystalline compounds, which can be satisfactorily recrystallised from dry solvents, and have fairly sharp, reproducible melting points.

Wolfrom[4] reported that the phenylboronates of the pentoses dissociated on chromatography using an aqueous organic solvent. A sample of pure D-arabinose was isolated from D-arabinose-bis phenylboronate, which was hydrolysed during chromatography in an aqueous solvent. It has been found that all the other available phenylboronates can be similarly hydrolysed, and in all cases when the compounds are identified on the chromatogram, by spraying with a suitable reagent, no streaking of the polyol is evident.

If a phenylboronate remained undissociated on chromatography in a certain solvent, one would expect that its R_F value would be considerably greater than that of the polyol, because of the smaller number of hydroxyl groups, and the presence of phenyl groups in the phenylboronate.

If, under the conditions of the hydrolysis, the equilibrium shown in Eqn. 4 were to the left, i.e. largely undissociated phenylboronate, then initially, the phenylboronate would be separated from the small amount of polyol, with a much lower R_F value.



(4)

The separated phenylboronate would then be again partially hydrolysed, and again the undissociated phenylboronate removed from the polyol.

Repetition of this process would leave the polyol spread from a position with the R_F value of the polyol, to a position with R_F value approaching that of the phenylboronate.

That this "streaking" does not occur, indicates that the phenylboronates are completely hydrolysed, immediately they come into contact with the aqueous solvent.

Paper chromatography using non-aqueous solvents has been described.[22] Chromatography paper was dipped in a solution of dimethyl sulphoxide in benzene (20%) and dried for a few minutes at 60°C. The phenylboronates were placed on the paper in chloroform solution, and the chromatogram developed with di-isopropyl ether, saturated with dimethyl sulphoxide. The only compounds which could be detected had an R_F value of 0, indicating that even under these conditions, the phenylboronates had been hydrolysed (perhaps by water in the paper) and the compounds detected were the polyols, which would be expected to have zero R_F values in this solvent (Expt. 37).

Thus, although the phenylboronates of polyols are stable in air, and to heat, they are rapidly hydrolysed by aqueous organic solvents.

INVESTIGATIONS INTO THE STRUCTURES OF CERTAIN PHENYLBORONATES

INVESTIGATIONS INTO THE STRUCTURES OF THE PHENYLBORONATES OF CERTAIN POLYOLS

Attempts have been made, by conventional chemical methods, to determine the structures of several phenylboronates. The galactitol, glycerol and D-glucose compounds were studied first, as these all contain at least one free hydroxyl group. Attempts were also made, to determine the structure of D-mannitol trisphenylboronate by chemical methods, and by physical measurements (namely infrared studies).

Thus, the initial aim was to find a reagent which would react with a free hydroxyl group in a phenylboronate without destroying the cyclic phenylboronate ester. The reagent must be such that when the phenylboronate esters are hydrolysed, the substituted group remains in the position to which it was originally attached.

The substituted polyol thus obtained can be identified, either by comparison with a known compound, or by structural investigations by standard techniques.

It has previously been shown [4] (~~Expt. 4~~) that phenylboronate esters are unstable in aqueous organic solvents. This fact introduces an

additional difficulty into the substitution reactions which can be used, in that anhydrous conditions are essential. However, the ease of hydrolysis of the phenylboronate esters facilitates the isolation of the substituted polyols required.

General Methods used for the Substitution of free Hydroxyl Groups

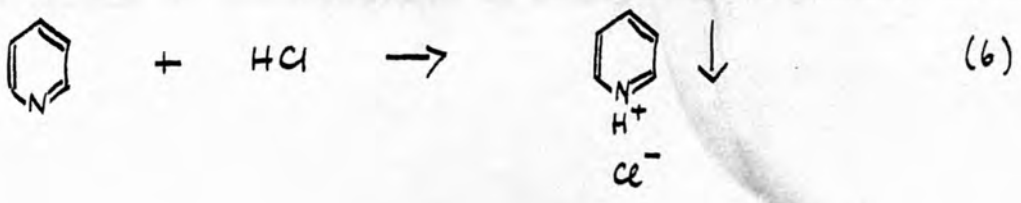
1. Reaction of Benzoyl Chloride

The use of benzoyl chloride as a reagent for reaction with free hydroxyl groups is a standard technique in organic chemistry.[23]

$$-OH + \phi\text{-}COCl \rightarrow -O\text{-}CO\text{-}\phi + HCl \qquad (5)$$

The reaction is carried out in an alkaline medium so that the HCl is removed as it is formed.

When benzoylation is used as a method of making a derivative of a phenol, the reaction is carried out in sodium hydroxide solution,[23] but in many other cases pyridine is used as the solvent.[24] In this case, evidence of reaction is given by the precipitation of pyridine hydrochloride.

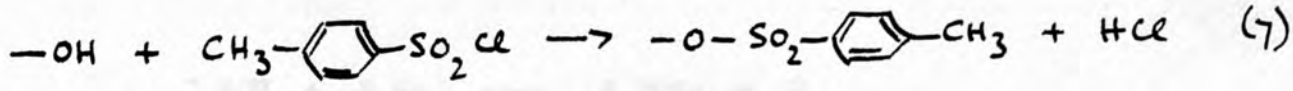


(6)

Benzoyl derivatives are in most cases insoluble in water. Those prepared in sodium hydroxide solution are precipitated from the solution. When pyridine is the solvent, the derivative is usually precipitated when the pyridine solution is poured into dilute aqueous acid.

2. Reaction of toluene-p-sulphonyl chloride

Another commonly used reagent for substituting free hydroxyl groups in polyols is toluene-p-sulphonyl chloride (tosyl chloride).[24] This reacts in a similar way to benzoyl chloride and is used in pyridine solution.



(7)

As in the case of benzoylation, reaction is indicated by the precipitation of pyridine hydrochloride (Eqn. 6). Tosylation is usually carried out at 5°C,[24] but in cases where substitution is difficult, refluxing in pyridine has been reported.[25]

3. Reaction of Methyl Iodide

Methylation is a technique which is commonly used in the investigation of the structures of polyhydroxy-compounds. Several

methods have been used. Purdie's[26] method used methyl iodide as both solvent and methylating agent, with silver oxide added to the solution. The disadvantages of this method are the high cost of the reagents and the insolubility of many sugar derivatives in methyl iodide, so that a number of treatments with the reagent is necessary for complete methylation. Haworth's method,[27] using dimethyl sulphate and 30% sodium hydroxide solution, has been used for sugars soluble in water. Methyl derivatives of carbohydrates have been formed via the sodio-derivative[28] using liquid ammonia as the solvent for the preparation of this derivative. After removal of the ammonia, the sodio-derivative is suspended in an inert solvent and methylated with methyl iodide.

$$\begin{aligned} 2\ ROH + 2\ Na &\longrightarrow 2\ RONa + H_2 \\ RONa + CH_3I &\longrightarrow R\text{-}OCH_3 + NaI \end{aligned} \qquad (8)$$

Recently, the most commonly used methylation technique has been that of Kuhn.[29] In this method, the compound to be methylated is dissolved in dimethylformamide and treated with methyl iodide and silver oxide. When sucrose is methylated in this way, one methylation only is required, whereas by other methods, several treatments would be necessary.

$$-OH + CH_3I \longrightarrow -O-CH_3 + HI$$
$$2HI + Ag_2O \longrightarrow 2AgI + H_2O \qquad (9)$$

Evidence of reaction is provided by the precipitation of yellow silver iodide.

4. Reaction of Phenylisocyanate

Phenyl isocyanate is a reagent which is used for the characterisation of alcohols,[30] and it forms a solid derivative with such a low molecular weight compound as methyl alcohol.[30] When it is used to characterise liquid alcohols, the two liquids are mixed together and reaction occurs immediately.

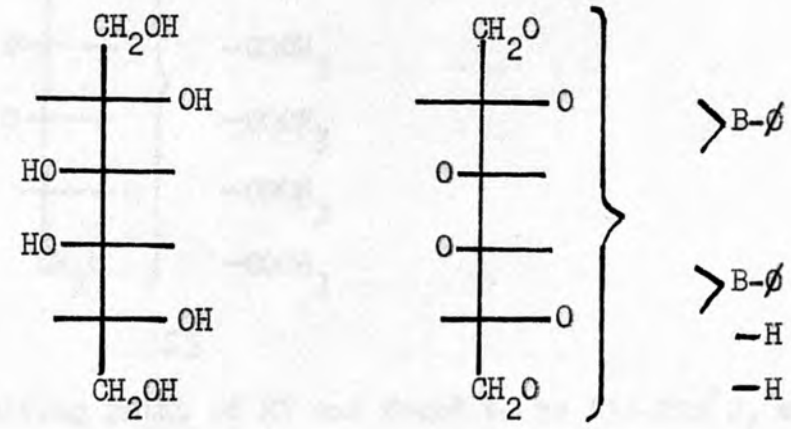
In contrast to the other reagents described, there is no elimination of water during the course of the reaction.

$$-OH + \phi.NCO \longrightarrow -O.CO.NH.\phi \qquad (10)$$

Phenyl isocyanate has been used as a reagent for substitution free hydroxyl groups in polysaccharides, using dimethyl formamide as solvent.[31]

The Elucidation of the Structure of Galactitol Bisphenylboronate

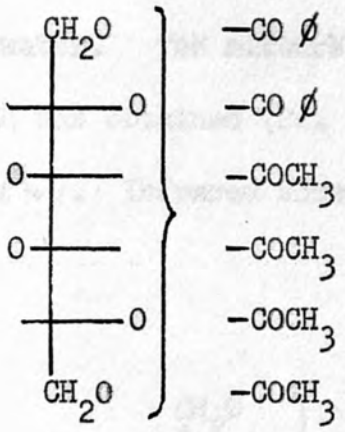
The preparation of a compound E, galactitol bisphenylboronate (Fd. C 59.9% H 6.0% B 6.3% Calc. for $C_{18}H_{20}O_6B_2$ C 60.0% H 5.6% B 6.2%), from galactitol (XLIV) and phenylboronic acid has been described (p.37 Expt. 4).



Galactitol XLIV E

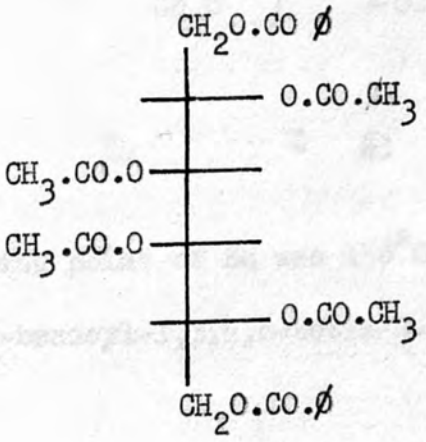
A portion of the compound E was treated with benzoyl chloride, in pyridine, according to standard procedure.[24] After the pyridine hydrochloride had been removed, the solution was poured into water, and dilute acid was added. The white precipitate obtained was treated with an acetylating mixture (acetic anhydride/glacial acetic acid/conc. H_2SO_4, 35:15:1) which is known to replace benzylidene groups by acetyl groups.[24]

On pouring this acetylating mixture on to ice, a white solid was produced. This solid, E3 (Fd. C 59.5% H 5.4% Calc. for $C_{28}H_{30}O_{12}$, C 60.2% H 5.4%) was shown to have no free hydroxyl groups (IR evidence). (Expt. 14).



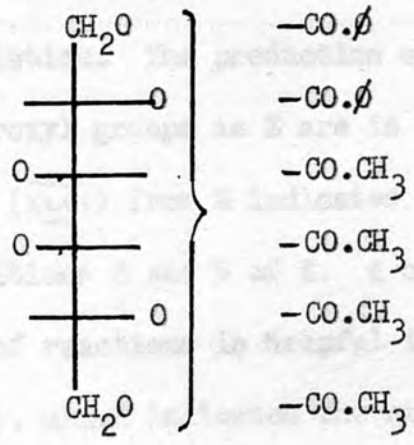
E3

The melting point of E3 was found to be 218-220°C, which indicates that it is 1,6-di-O-benzoyl-2,3,4,5-tetra-O-acetyl-galactitol (XLV).[24]



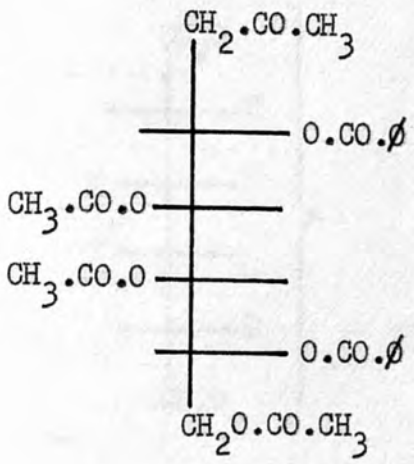
XLV

A second portion of E was treated with benzoyl chloride as before. However, in this case, after the removal of the pyridine hydrochloride, the remaining pyridine was removed at reduced pressure, until a thick syrup remained. This residue was treated directly with the acetylating mixture, without any contact with water. The mixture was then poured on to ice and a white solid E4 was obtained (Fd. C 59.6% H 5.7% Reqd. for $C_{28}H_{30}O_{12}$, C 60.2% H 5.4%). Infrared showed an absence of hydroxyl groups (Expt. 15).



E4 ≡ E3

The melting point of E4 was 158°C, which indicated that it is 2,5-di-O-benzoyl-1,3,4,6-tetra-O-acetyl-galactitol (XLVI)[24]

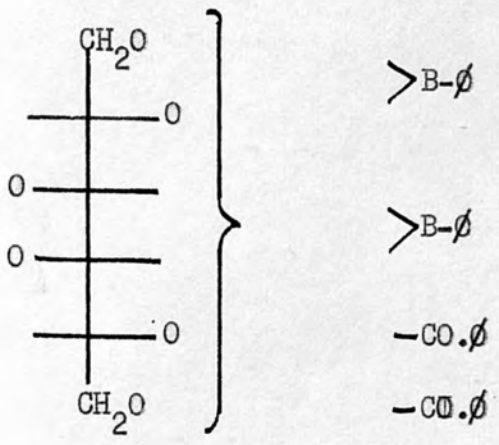


XLVI

The two compounds L (XLV) and L (XLVI) are produced from the same starting material, E, both routes involving benzoylation, followed by acetylation. The production of (XLV) from E indicates that the free hydroxyl groups in E are in positions 1 and 6, whereas the production of (XLVI) from E indicates that the free hydroxyl groups are in positions 2 and 5 of E. A closer inspection of these two series of reactions is helpful in determining whether it is (XLVI) or (XLV), which indicates the structure of E.

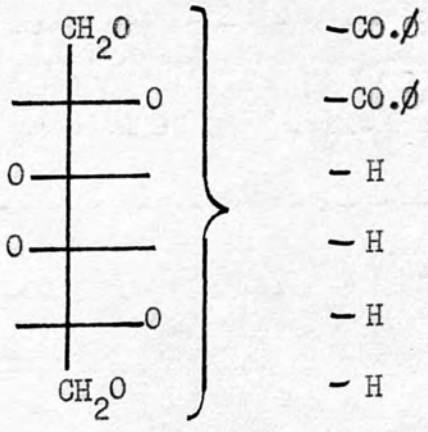
In the preparation of (XLV), water was introduced into the reaction mixture, between the benzoylation and acetylation reactions.

It can be postulated that the reaction of benzoyl chloride with E in pyridine solution gives a compound E1.



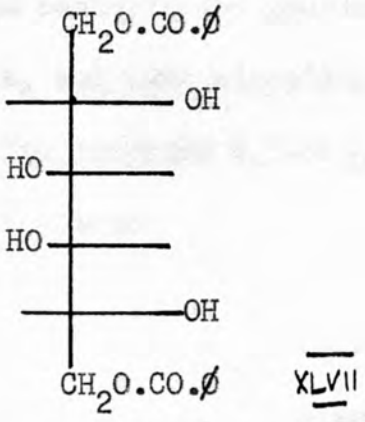
E1

When the pyridine solution is poured into dilute aqueous acid, the compound E1 will be in an aqueous organic medium. It is known that under these conditions phenylboronate esters are hydrolysed,[4] giving phenylboronic acid and a polyol. Thus it is probable that E1 is hydrolysed to E2, a di-O-benzoyl-galactitol.



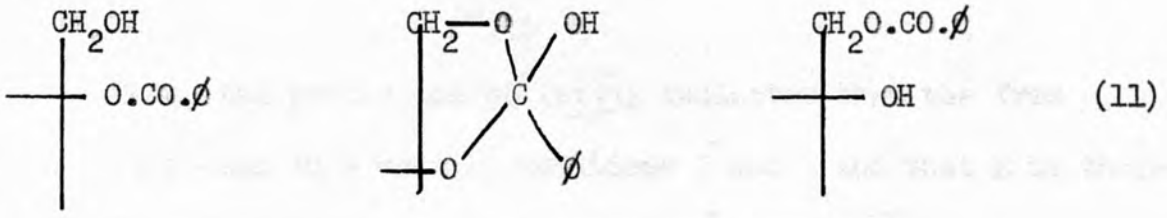
E2

It is likely that, whichever positions the benzoyl groups are initially attached to, they will move to the 1 and 6 positions, giving 1,6-di-O-benzoyl-galactitol (XLVII)



XLVII

The mechanism of the migration of a benzoyl group from a secondary to a primary hydroxyl group involves the formation of an ortho-ester, thus (Eqn. 11).[32]



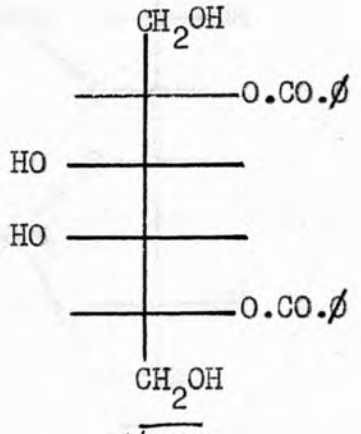
(11)

ortho-ester

Therefore, on the acetylation of E2, the compound (XLV)

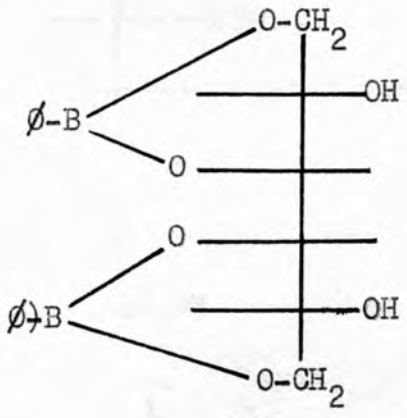
will be produced, irrespective of the initial positions of the free hydroxyl groups in E.

The production of (XLVI), in which acetylation follows immediately upon benzoylation precludes the formation of E2 by hydrolysis, and thus migration of the benzoyl groups is prevented. The compound 2,5-di-O-benzoyl-galactitol (XLVIII) has never been prepared.



XLVIII

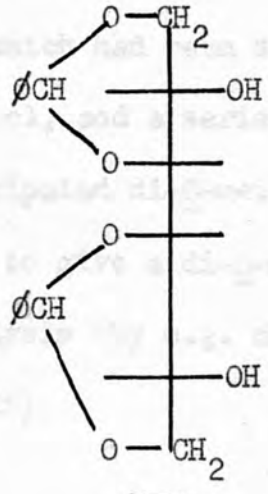
Thus, the production of (XLVI) indicates that the free hydroxyl groups in E were in positions 2 and 5 and that E is therefore probably galactitol-1,3:4,6-bisphenylboronate (XXVI).



XXVI

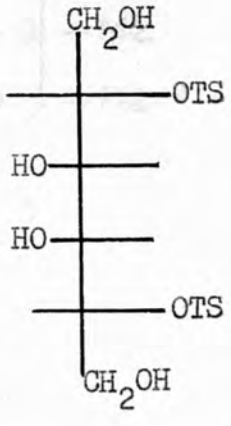
Confirmation of the structure (XXVI) for compound E was considered necessary, particularly as the yields of compounds (XLV) and (XLVI) were very low (< 10%).

Compound E was also treated with tosyl chloride, in pyridine at 5°C, i.e. under the conditions utilised for the tosylation[24] of 1,3:4,6-di-O-benzylidene-galactitol (XLIX).

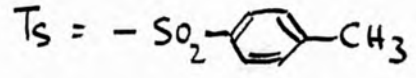


XLIX

No 2,5-di-O-tosyl galactitol (L) was obtained on pouring the mixture into water (Expt. 16).

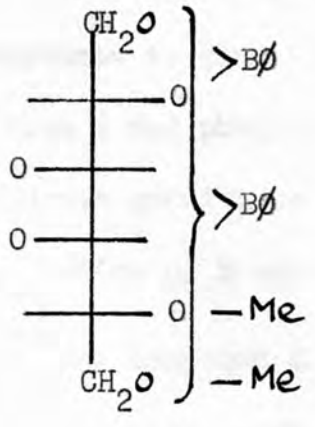


L

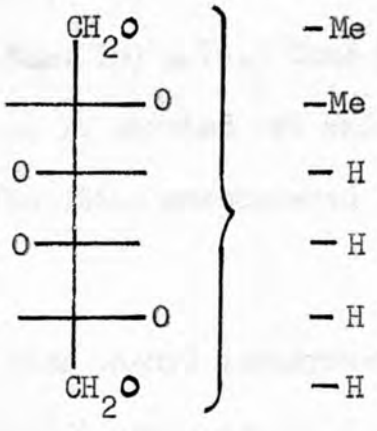


The conditions were varied, and no appreciable reaction occurred after the temperature was raised to that of the boiling point of pyridine (115°C) (Expt. 16). Chromatography in solvent (α) indicated only very small quantities of compounds with R_F values greater than that of galactitol.

Methylation of E was attempted by Kuhn's method (Expt. 17). Chromatographic analysis of the reaction mixture (E, MeI, & Ag_2O in dimethylformamide) which had been shaken overnight, revealed a small quantity of galactitol, and a series of methyl ethers of galactitol, instead of the anticipated di-O-methyl-galactitol. Methylation of E would be expected to give a di-O-methyl-galactitol bisphenylboronate (E5) which on hydrolysis (by e.g. chromatography) would give a di-O-methyl galactitol (E6).



E5



E6

The production of a series of methyl ethers of galactitol instead of E6, may be due to the nature of the reaction involving an hydroxyl group, methyl iodide, and silver oxide. As previously described (Eqn. 9) water is produced.

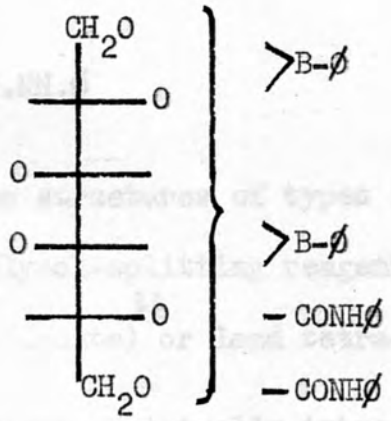
$$\begin{aligned} -OH \; + \; MeI \; &\longrightarrow \; -OMe + HI \\ 2HI \; + \; Ag_2O \; &\longrightarrow \; 2AgI \; + \; H_2O \end{aligned} \qquad (9)$$

It is possible that this water hydrolyses the phenylboronate esters - producing partially methylated galactitol and galactitol itself. The liberated polyol could then give rise to a series of methyl ethers of galactitol. Thus methylation is not a satisfactory method for determining the structure of E.

No water is produced when an alcohol reacts with phenyl isocyanate to give a urethane. (Eqn. 10) p.56. Thus the reaction between E and phenyl isocyanate can be carried out under strictly anhydrous conditions and the difficulties encountered in the methylation of E can be avoided.

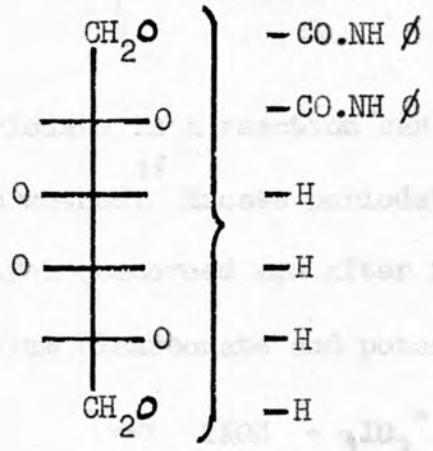
The compound E was treated with phenyl isocyanate in benzene at the boiling point of benzene (80°C) and a crystalline product, E7, m.p. 223-224°C was obtained (with analysis figures Fd. C 64.7%;

H, 5.3%; N, 4.8%. Calc. for $C_{32}H_{30}N_2O_8B_2$ (galactitol bisphenylboronate bisurethane) C, 64.8%; H, 5.1%; N, 4.7%). (Expt. 18)



E7

The galactitol bisurethane E8 was obtained by hydrolysis in a dioxan/water mixture. It had m.p. 257°C. Fd. C, 56.8; H, 5.6; N, 6.6. Calc. for $C_{20}H_{22}N_2O_8$, C, 57.0; H, 5.7; N, 6.7).



E8

The possible structures of a galactitol bisurethane E8 are shown by structures (LI)-(LIX) (p.69).

where

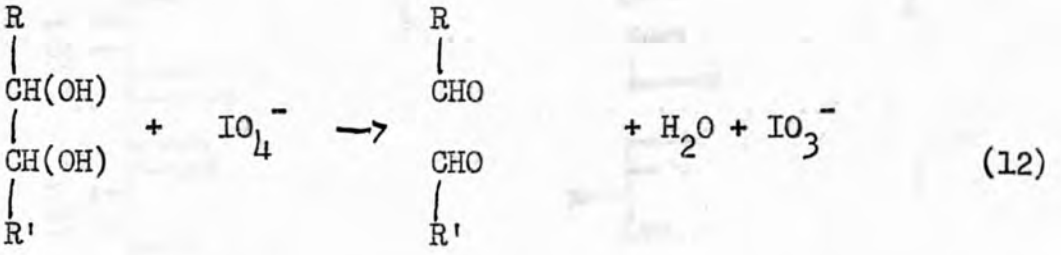
—• is CH_2OH

— is OH

—X is O.CO.NH.Ø

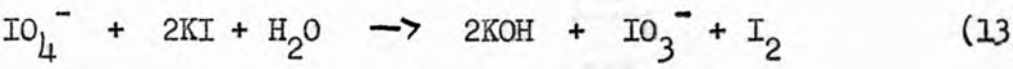
Distinctions between structures of types (LI)-(LIX) can often be made by the use of a glycol-splitting reagent such as periodic acid (or sodium metaperiodate)[33] or lead tetra-acetate.[34]

Periodic acid oxidation, originally introduced by Malaprade,[33] is applicable to compounds containing vicinal hydroxyl groups (or hydroxyl and amino groups on adjacent carbon atoms) (eqn. 12).

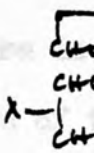
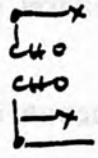
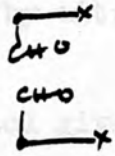
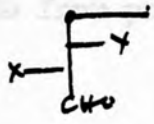
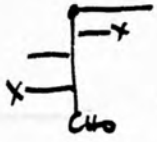
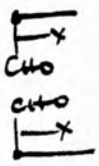
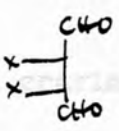


(12)

The uptake of periodate in a reaction can be estimated by the iodine and arsenite method.[36] Excess periodate is added to the solution of the polyol concerned and after reaction, the mixture is neutralised with sodium bicarbonate and potassium iodide is added.



(13)

Structure	mol. IO_4^- consumed	other compounds	mol. HCO_2H	mol. HCHO
LI	3		2	1
LII	2		1	1
LIII	2		0	1
LIV	2		1	0
LV	3		2	0
LVI	2		1	1
LVII	1		0	1
LVIII	1		0	0
LIX	2		0	2

An excess of standard arsenite solution is added, which reacts with the iodine.

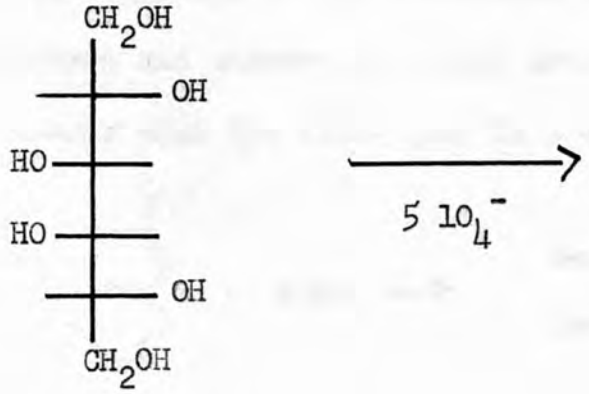
$$ASO_3^- + I_2 + 2HCO_3^- \longrightarrow ASO_4^- + 2I^- + H_2O + 2CO_2 \qquad (14)$$

The excess of standard arsenite solution, after this reaction, can be estimated by titration with standardised iodine solution.

The uptake of periodate can also be estimated spectrophotometrically by measuring the decrease in absorption of the periodate ion at 222.5 mμ.[36] The absorption of the iodate ion has to be considered. This is a very sensitive method.

The formic acid produced by the secondary hydroxyl groups of a molecule can be estimated directly by titration to pH 6 with alkali.[37]

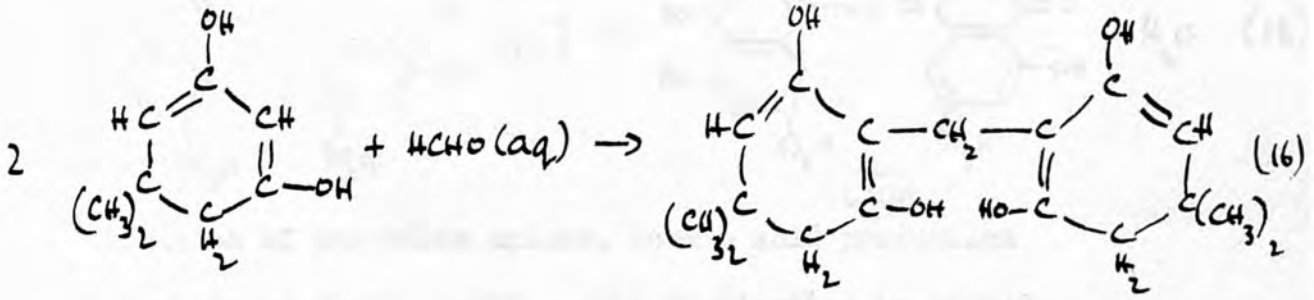
Periodate oxidation of galactitol gives 4 mol.HCO_2H from secondary-OH groups and 2 mol. HCHO from primary-OH groups.



(15)

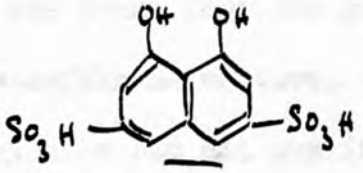
Formaldehyde can be estimated gravimetrically by the dimedone method.[38] A concentrated solution of dimedone ($\overline{LX}$) in acetone is

added to the formaldehyde solution and the precipitate (LXI) is collected and weighted.



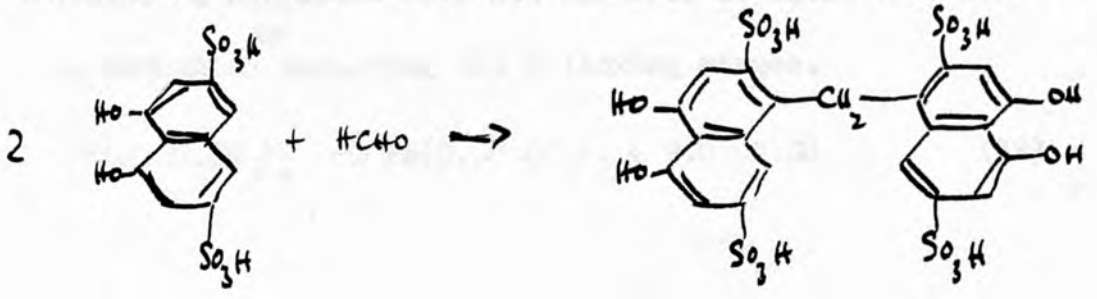
LX LXI (16)

Formaldehyde gives a purple colour with chromotropic acid (1,8-dihydroxy-naphthalene-3,6-disulphonic acid LXII) in the presence of conc. sulphuric acid, and HCHO can be estimated colorimetrically, depending on this reaction.[39]



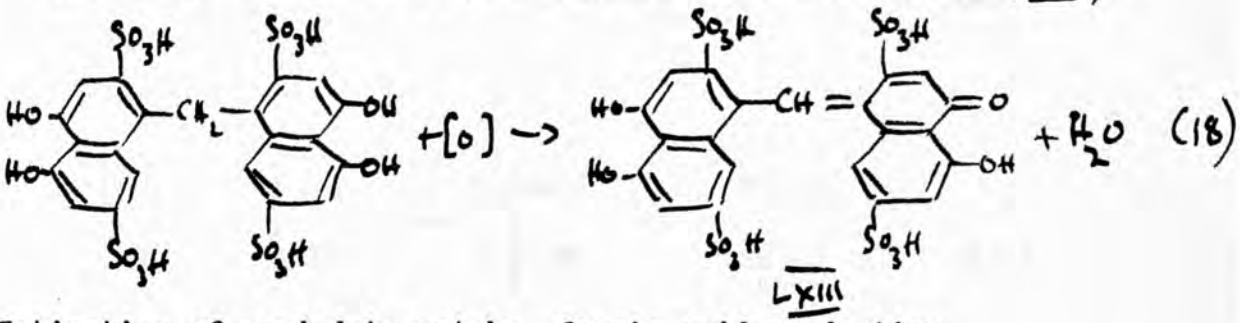
LXII

The chemistry of the production of the purple colour from formaldehyde and chromotropic acid is not fully understood. It is likely that the first step is a condensation as in Eqn. 17.



LXII (17)

This is probably followed by oxidation to a p-quinoidal compound (Eqn. 18) - which is responsible for the purple colour (LXII)



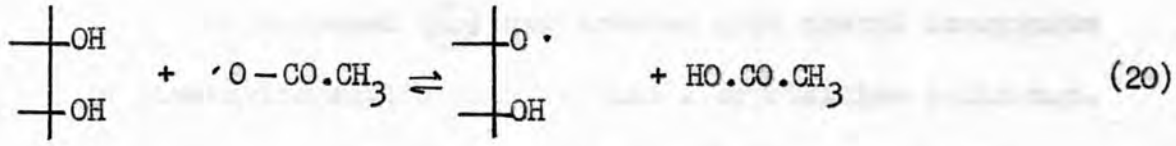
(18)

Estimation of periodate uptake, formic acid production and formaldehyde production will enable distinction to be made in structures (LI)-(LIX), except for (LII) and (LVI). Distinction between (LV) (a 1,6-substituted galactitol) and (LVII) (a 2,5-substituted galactitol) is obvious.

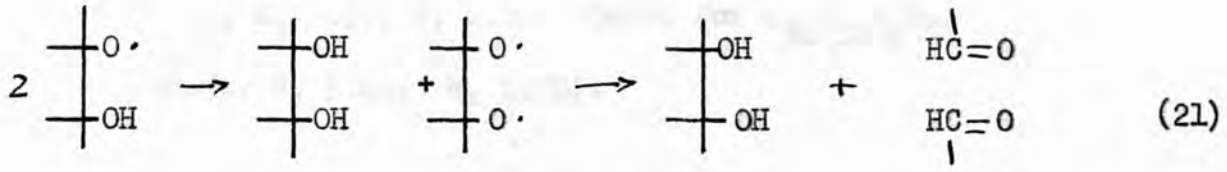
An aqueous solution of the compound to be studied, is required, and it was found that the galactitol bisurethane, E8, was insoluble in a water/dioxan mixture. Thus oxidation of the compound by sodium metaperiodate was not possible (Expt. 19).

Lead tetraacetate, also a glycol splitting reagent,[34] is usually used in organic solvents, such as glacial acetic acid, or benzene. A suggested reaction sequence is based on a free radical mechanism[40] involving the following stages.

$$Pb(O.CO.CH_3)_4 \longrightarrow Pb(O.CO.CH_3)_2 + 2.O.CO.CH_3 \qquad (19)$$



(20)



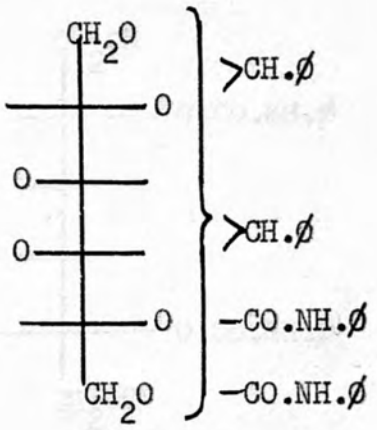
(21)

When E8 was treated with lead tetraacetate in acetic acid solution, it was found that the urethane groups were removed by the acid, leaving galactitol (XLIV) (Expt. 20). An attempt to carry out the reaction in dioxan solution was unsuccessful as the lead tetraacetate reacted immediately with the dioxan to give a yellow precipitate (Expt. 20). E8 was not soluble in benzene, and oxidation could not be carried out in that solvent.

Since the elucidation of the structure of E8 was not possible by degradative methods, an attempt was made to synthesise a galactitol-2,5-bisurethane, by an unambiguous route.

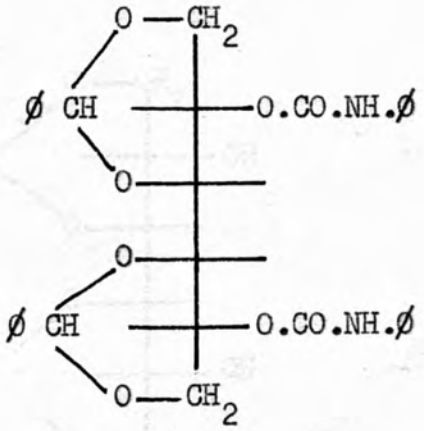
1,3:4,6-di-O-benzylidene-galactitol (XLIX) was prepared from galactitol (XLIV) by the method of Haskins, Hann and Hudson[24], i.e. by treating galactitol (XLIV) with benzaldehyde in the presence of dry HCl (Expt. 21).

The compound (XLIX) was treated with phenyl isocyanate in dimethylformamide solution and a crystalline solid m.p. 343°C was produced (Expt. 22). This compound M1 has C 68.2%; H, 5.3;; N, 4.8%. Calc. for $C_{34}H_{32}N_2O_8$. C, 68.5%; H, 5.4%;, N, 4.7%).



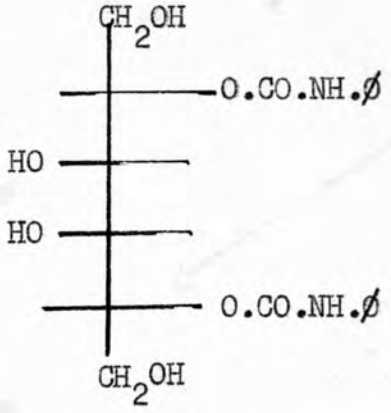
M1

Derived from the compound (XLIX) it can be only the 1,3:4,6-di-O-benzylidene-2,5-di-urethane-galactitol (LXIV).



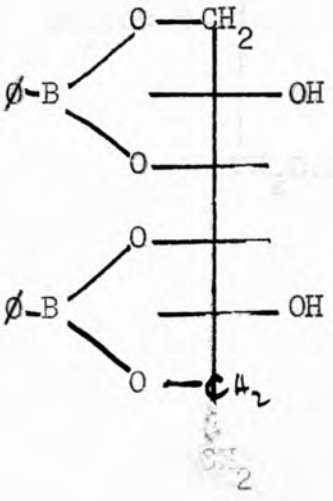
LXIV

The compound (LXIV) was treated with an acid resin,[41] and a white compound produced, m.p. 257°C. Analysis figures, Fd. C, 56.8%; H, 5.6%; N, 6.6%. Calc. for $C_{20}H_{22}N_2O_8$. C, 57.0%; H, 5.7%; N, 6.7%. Derived from (LXIV) this compound can only be (LVIII).

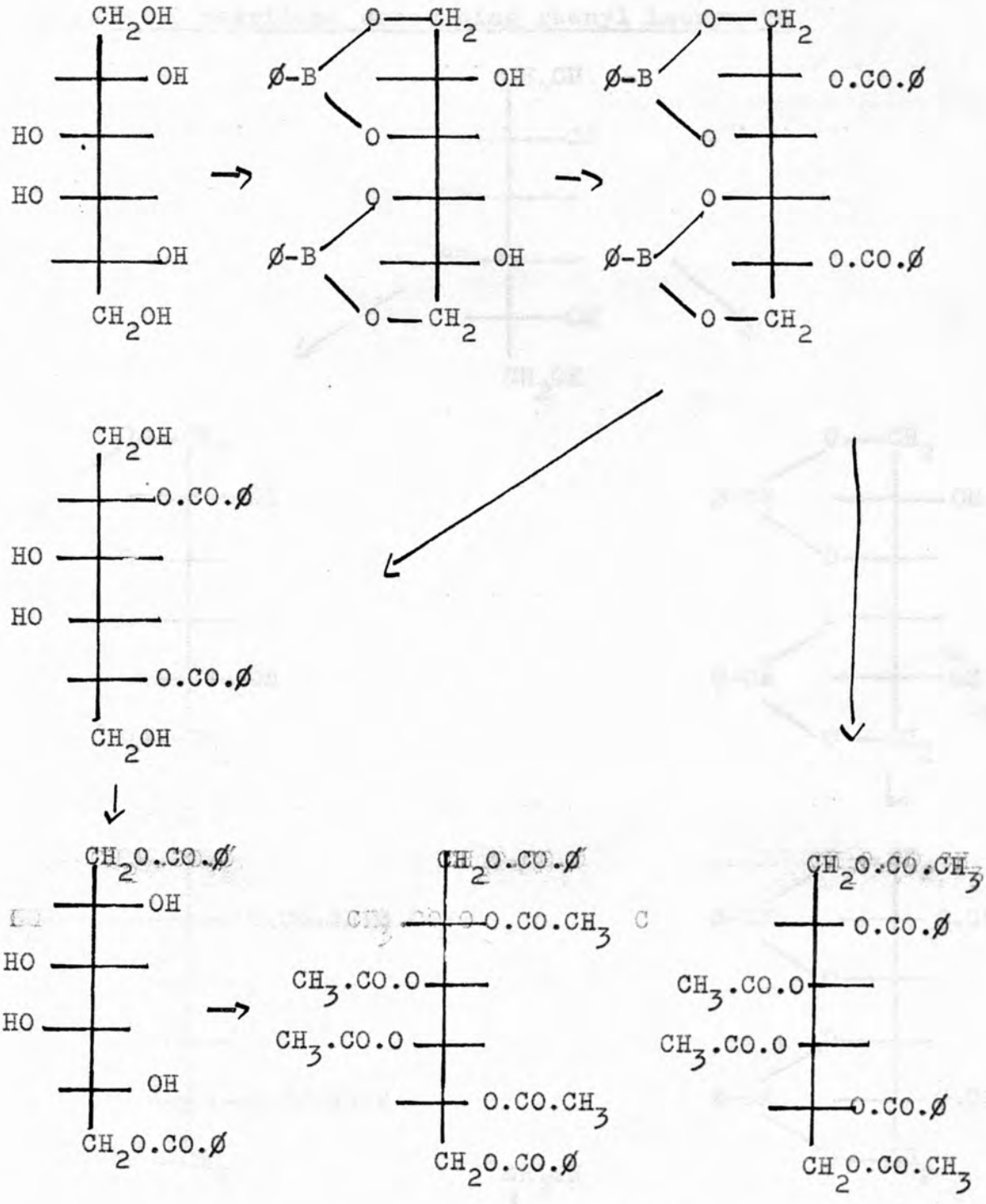


LVIII

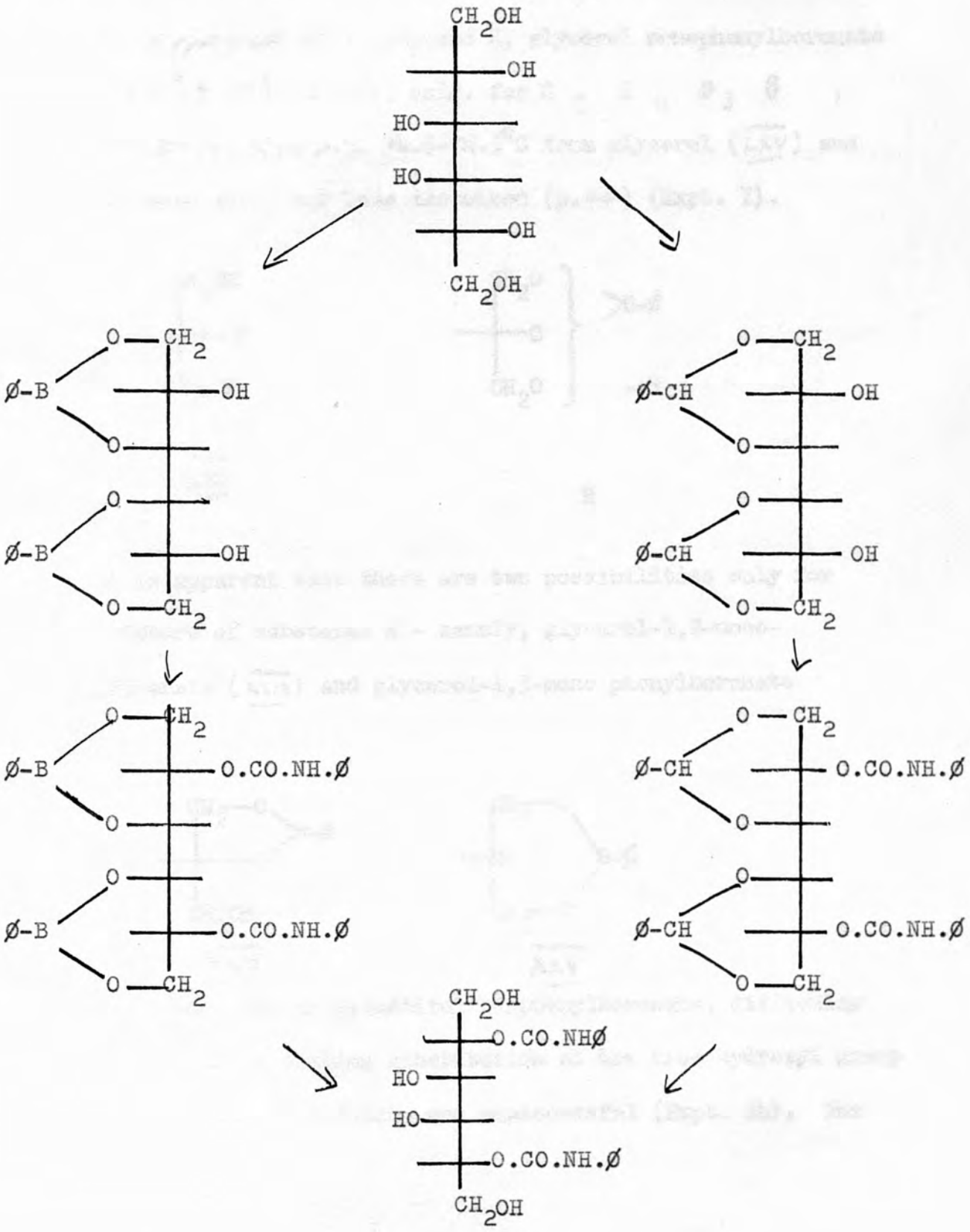
Thus, the structure of galactitol bisphenylboronate must be the 1,3:4,6-bisphenylboronate of galactitol, as indicated by the benzoylation reactions with the compound.



Sequence of reactions concerning benzoyl chloride

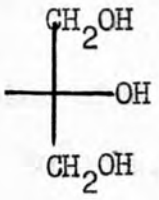


Sequence of reactions concerning phenyl isocynate

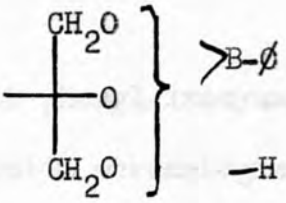


The elucidation of the structure of glycerol monophenylboronate

The preparation of a compound H, glycerol monophenylboronate (Fd. C 60.0% H 6.1% B 6.0%, calc. for $C_9H_{11}O_3B$, C 60% H 6.2% B 6.2%) with m.p. 74.5-76.5°C from glycerol (LXV) and phenylboronic acid, has been described (p.40) (Expt. 7).

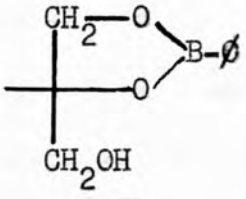


LXV

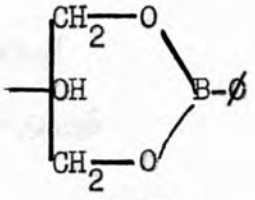


H

It is apparent that there are two possibilities only for the structure of substance H - namely, glycerol-1,2-monophenylboronate (XXIX) and glycerol-1,3-mono phenylboronate (XXX).



XXIX

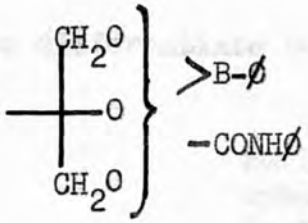


XXX

As in the case of galactitol bisphenylboronate, difficulty was experienced in causing substitution of the free hydroxyl group in the compound. Tosylation was unsuccessful (Expt. 24). The

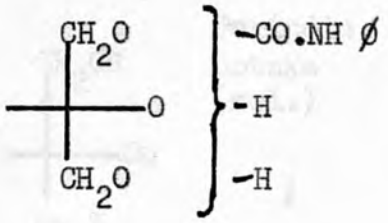
compound H was treated with MeI and Ag_2O in dimethylformamide solution, and the mixture was shaken overnight.[29] Chromatography of the reaction mixture in solvent (α) revealed a small amount of a component with an R_F value greater than that of glycerol (probably a mono-methyl glycerol) (Expt. 25). It was obvious that methylation was not a satisfactory method for determining the structure of H.

A portion of H was treated with phenyl isocyanate, in benzene solution at 80°C (b.p., benzene), until chromatography of the reaction mixture revealed that all of H had reacted with the isocyanate (Expt. 26). A crystalline solid (Hl) was obtained, m.p. 117°C, which had an infrared spectrum showing no hydroxyl group absorption. Elemental analysis showed C 64.5% H 5.3% N 4.9% Calc. for $C_{16}H_{16}NO_4B$, C, 64.5; H, 5.4; N, 4.7 - a glycerol-monophenylboronate-monourethane.



Hl

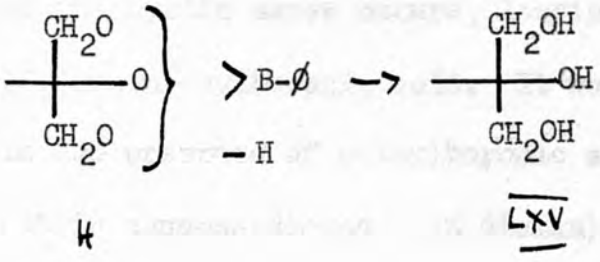
An attempt to isolate a glycerol-monourethane was made.



H2

Hydrolysis of H1 was effected by chromatography in solvent (a) and the monourethane H2 was eluted from the paper in methanol solution (Expt. 27). H2 was found to be an oil, which could not be distilled without decomposition. Chromatographic analysis of the distillate showed that some glycerol (LXV) was present after decomposition. When a solution of phenylboronic acid in methanol was added to the distillate, a sample of glycerol monophenylboronate, m.p. 74.5-76.5°C (H) was obtained (Expt. 28).

There are two possible structures (LXVI) and (LXVII) for the compound H1, and hence two structures for H2 (LXVIII) and (LXIX). It should be possible to differentiate between (LXVIII) and (LXIX) by periodate oxidation.

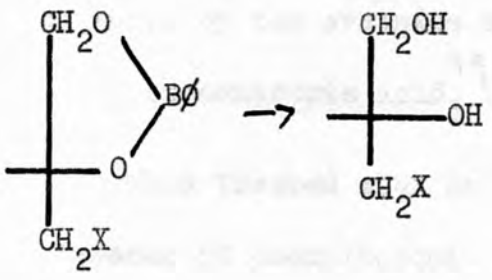


H

LXV

Periodic uptake (mol.)		HCHO (mol.)
2	HCO_2H	2

X = O.CO.NH.Ø

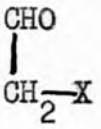


LXVI

LXVIII

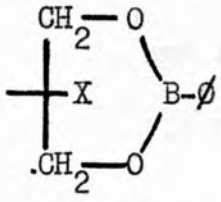
Periodic uptake (mol.) 1

products

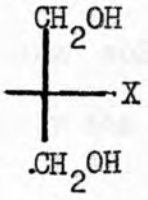


LXV

HCHO (mol.) 1

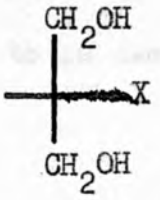


LXVII



LXIX

0



LXIX

0

As it was not possible to purify the glycerol-monourethane H2, an accurate determination of the periodate uptake of the molecule could not be carried out directly.

It has been shown that when glycerol monophenylboronate monourethane (H1) is dissolved in an aqueous organic solvent, hydrolysis of the cyclic ester occurs, leaving in solution glycerol monourethane, H2, and phenylboronic acid. It was decided to oxidise H2 with periodate in the presence of phenylboronic acid. The oxidations were carried out in aqueous dioxan (25% dioxan) (Expt. 29). Periodate uptake was

measured by the arsenite method[35] and formaldehyde production by using chromotropic acid.[39] (Expt. 30).

When treated with an excess of periodate, glycerol, in the presence of phenylboronic acid consumed in 300 min., 2.8 mol. of periodate. (Fig. 14). p.83 Theoretically glycerol consumes 2 mol. periodate. The difference between this value and the consumption of 2.8 mol. periodate by glycerol in the presence of phenylboronic acid, and in dioxan solution was thought to be due to the phenylboronic acid, and possibly the dioxan.

Certain discrepancies have been found in the periodate oxidation of polyols in phosphate buffer at pH 7.5[42] and in borate solution at pH 10.[43] Therefore it was decided to investigate the effect of phenylboronic acid, in 25% dioxan solution, on periodate. When treated with an excess of periodate, phenylboronic acid showed an apparent consumption of 0.8 mol. periodate in 300 min. The consumption of periodate in this reaction was slow and steady and may be due to the action of periodate on dioxan, or may be due to the action of periodate on phenylboronic acid.

When this value (0.8) is subtracted from that obtained for glycerol under the same conditions (2.8) the expected value for glycerol of 2 mol. is obtained.

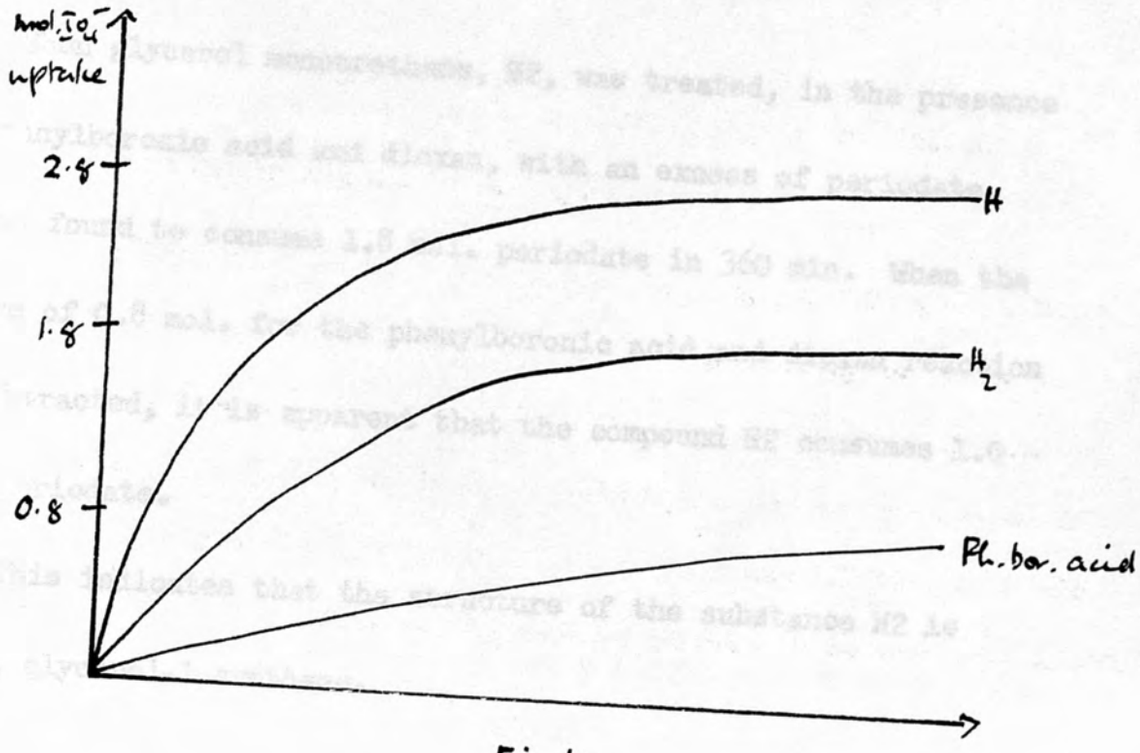


Fig. 4

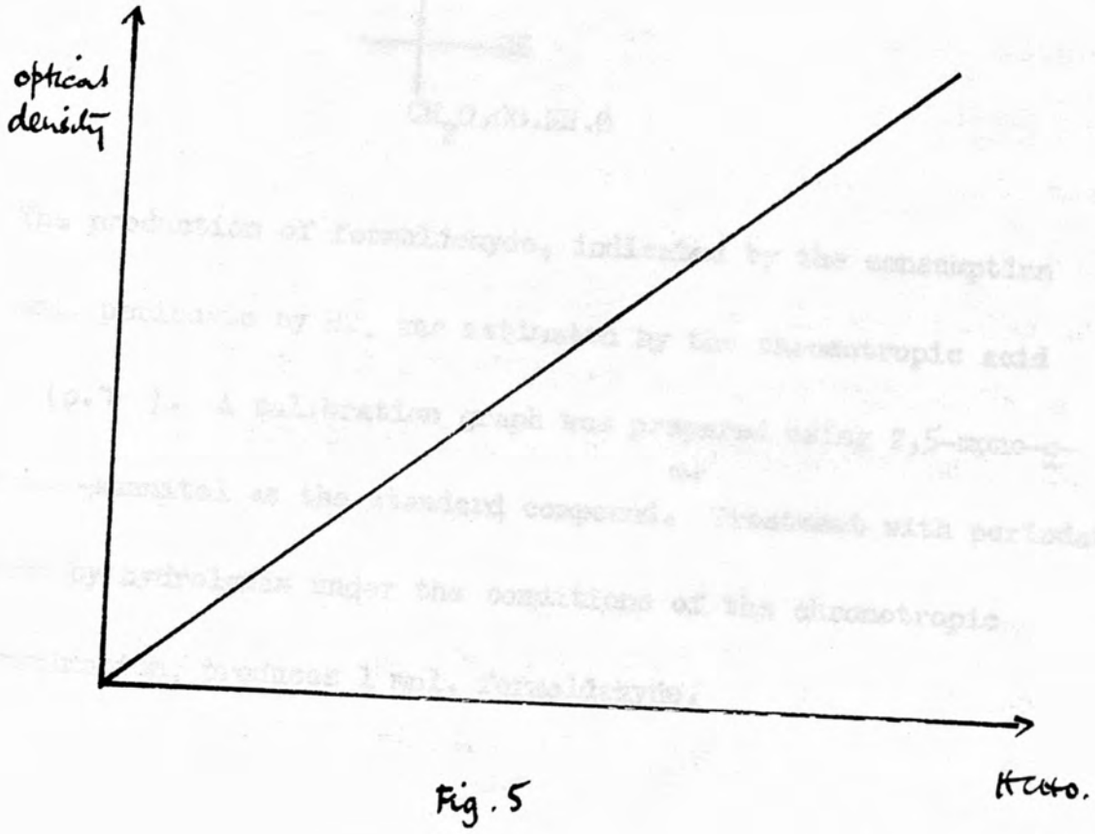
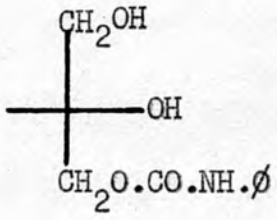


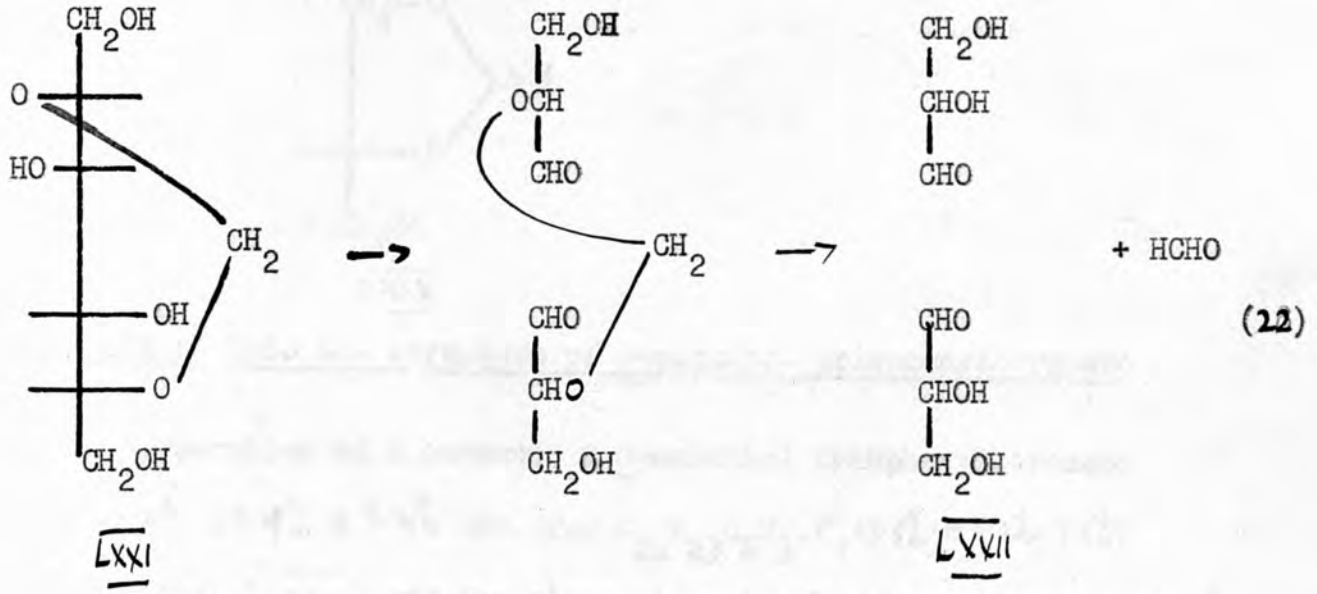
Fig. 5

When glycerol monourethane, H2, was treated, in the presence of phenylboronic acid and dioxan, with an excess of periodate, it was found to consume 1.8 mol. periodate in 360 min. When the figure of 0.8 mol. for the phenylboronic acid and dioxan reaction is subtracted, it is apparent that the compound H2 consumes 1.0 mol. periodate.

This indicates that the structure of the substance H2 is (LXVIII), glycerol-1-urethane.



The production of formaldehyde, indicated by the consumption of 1 mol. periodate by H2, was estimated by the chromotropic acid method (p.71). A calibration graph was prepared using 2,5-mono-o-methylene-mannitol as the standard compound[44]. Treatment with periodate, followed by hydrolysis under the conditions of the chromotropic acid estimation, produces 1 mol. formaldehyde.



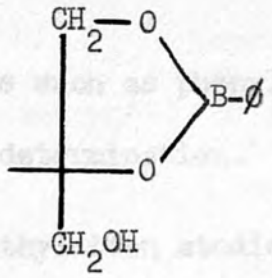
(22)

Glyceraldehyde (LXXII) gives a slight yellow colour with chromotropic acid, but at the wavelength used for measurements, this does not interfere with the formaldehyde estimation.

It was found that during the consumption of 1 mol. periodate by substance H2, that 1 mol. formaldehyde was produced. This confirms the previous conclusion that the structure of H2 is (LXVIII).

A portion of the periodate oxidised solution of H2 was treated with a concentrated solution of dimedone in acetone.[38] A small quantity (not enough to determine quantitatively) of the formaldehyde derivative of dimedone was produced. (m.p. 190°C) thus confirming the production of formaldehyde by the oxidation of H2.

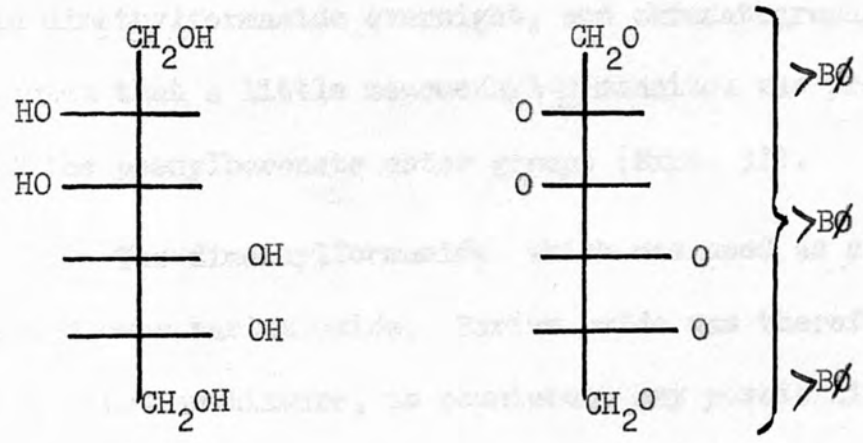
As the structure of H2 is (LXVIII), then that of H must be (XXIX) (p. 41), the 1,2-monophenylboronate of glycerol.



XXIX

Investigations into the structure of D-mannitol trisphenylboronate

The preparation of a compound A D-mannitol trisphenylboronate (Fd., C 62.9% H 4.9% B 7.4% Calc. for $C_{24}H_{23}O_6B_3$. C, 63.5%; H 5.2%; 7.5%) from D-mannitol (LXXIII) and phenylboronic acid, has been discussed (p.34 Expt. 1).



LXXIII

A

Possible structures for this compound have been discussed (p.36).

The absence of free hydroxyl groups, precluded the

use of reagents such as phenyl isocyanate, as the initial stage in structural determination.

From methylation studies on galactitol bisphenylboronate (Expt. 17, p.65) it seemed possible that the methylation reaction was causing breakdown of the cyclic phenylboronate esters. Theoretically, methylation of D-mannitol trisphenylboronate should have no effect unless the conditions of the reaction cause the breakdown of the molecule.

Compound A was treated with methyl iodide and silver oxide, in dimethylformamide overnight, and chromatography in solvent (α) showed that a little monomethyl-D-mannitol was present, after hydrolysis of the phenylboronate ester groups (Expt. 31).

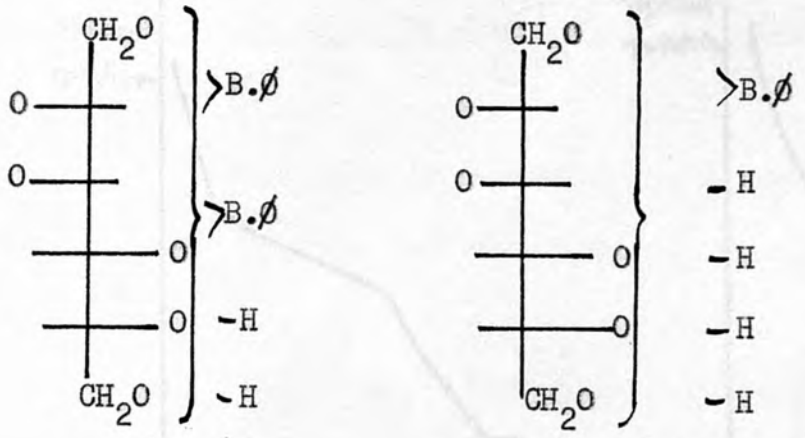
The dimethylformamide which was used as solvent, had been dried over barium oxide. Barium oxide was therefore incorporated into the reaction mixture, to counteract any possibilities of hydrolysis, particularly after any initial methylation (which produces water) has occurred. However, it was found that methylation was more rapid in the presence of the barium oxide than when it was omitted from the mixture (Expt. 31). It is possible that this is because barium oxide acts as an alkali in removing phenylboronate ester groups from the polyol.

Substitution of phenylboronate groups in compound A by methyl groups was attempted by using varying quantities of methyl iodide, and the results are summarised below (Expt. 32), in comparison with the results of similar reactions, using mannitol (LXXIII).

Compound	MeI Theoretical for	Compounds detected in solvent (a)	
		D-Mannitol	D-Mannitol derivatives
	2 OH	strong	strong mono-
(A)	6 OH	absent	strong di-
	15 OH	absent	trace di-
	2 OH	absent	absent
(LXXIII)	6 OH	absent	absent
	15 OH	absent	absent

The widely varying results obtained, depending on the conditions used, precluded the use of this method for a study of the structure of the compound A.

The partial hydrolysis of compound A to give the D-mannitol- bis- and mono-phenylboronates was attempted (A1 and A2).



A1 A2

The reaction was followed by measuring the optical rotation of a solution of the reactants (Expt. 33). The tris-ester, A, was dissolved in dioxan, and water added to the solution, in small volumes.

Considering the hydrolysis of 1 mol. of A by 3 mol. water, two reaction sequences are possible (Eqns. 23 and 24).

$$A \xrightarrow{H_2O} A1 \xrightarrow{H_2O} A2 \xrightarrow{H_2O} \underline{D}\text{-Mannitol} \qquad (23)$$

$$A \xrightarrow{3H_2O} \underline{D}\text{-Mannitol} \qquad (24)$$

If sequence 23 takes place then a graph of optical rotation against water added, should show three stages, equivalent to the three hydrolysis steps (Fig. 6).

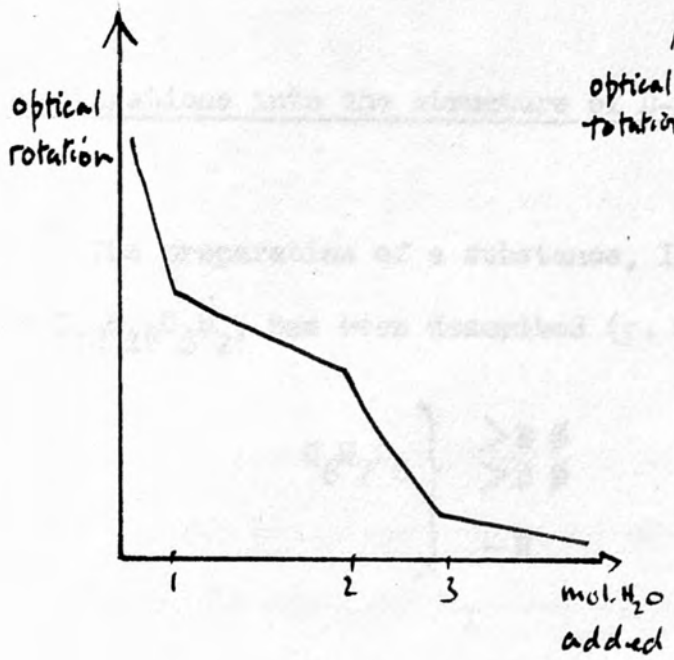


Fig. 6

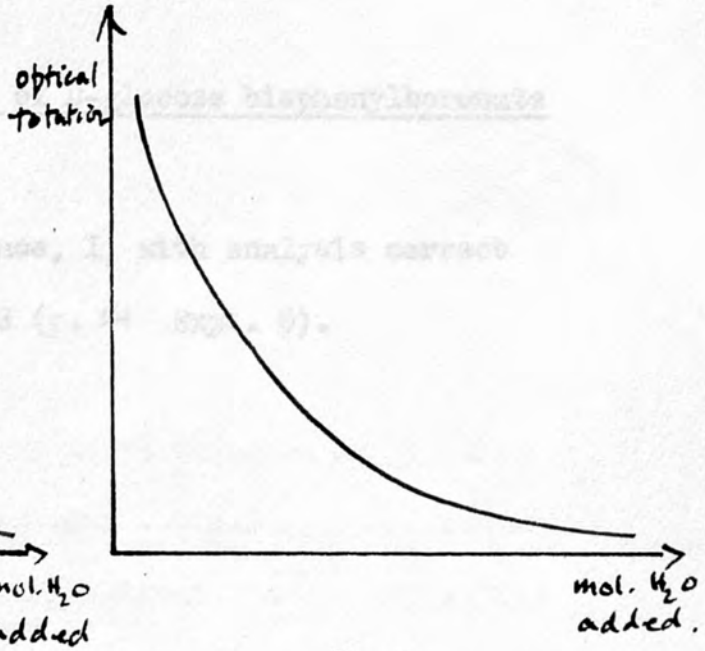


Fig 7.

Sequence 24 would result in a graph as in figure 7, and it was found that this type of hydrolysis did occur.

It seems probable that only physical measurements, e.g. infrared studies, will enable the detailed structure of mannitol tris-phenylboronate (A) to be determined.

Investigations into the structure of D-glucose bisphenylboronate

The preparation of a substance, I, with analysis correct for $C_{18}H_{18}O_6B_2$, has been described (p. 41 Expt. 8).

$$\left.\begin{matrix} C_6H_7O_6 \\ \\ \\ \end{matrix}\right\} \begin{matrix} >B\,\phi \\ >B\,\phi \\ -H \end{matrix}$$

I

This is consistent with a structure in which two phenylboronate rings are included in the molecule. Infrared analysis shows absorption at the hydroxyl group frequency.

However, the results of the attempted elucidation of the structure of this compound have produced a certain amount of conflicting evidence.

The compound I was treated, in dry benzene solution, with phenyl isocyanate (Expt. 34) and a solid was obtained. This had a m.p. 45°C and analysis figures. Fd. C, 63.4%; H, 5.9% and N, 9.2%. The values calculated for $C_{25}H_{23}O_7N\,B_2$, a D-glucose-bisphenylboronate monourethane II, are C, 63.6%; H, 4.9%; N, 3.0%.

$$C_6H_7O_6 \left\{ \begin{array}{l} >B\text{-}\phi \\ >B\text{-}\phi \\ -CO.NH.\phi. \end{array} \right.$$

II

The compound was treated with tosyl chloride in pyridine solution, and the results compared with a similar experiment using methyl α-D-glucopyranoside-4,6-phenylboronate (XII) (Expt. 35). Tosylation of (XII) should give methyl 2,3-di-O-tosyl-α-D-gluco-pyranoside (on hydrolysis) and on chromatography of the reaction mixture in solvent (a) a compound with R_F value 0.76 is evident. Chromatography of the reaction mixture in which I is treated with tosyl chloride shows one compound (besides D-glucose) with an R_F value of 0.69. The similarity of the R_F values, 0.69 and 0.76 suggests that the D-glucose derivative with R_F 0.69 may be a trisubstituted compound.

It is obvious that more evidence is required before any conclusion can be made about the structure of the compound I.

THE FORMATION OF 5- or 6-MEMBERED CYCLIC PHENYLBORONATE RINGS ON THE REACTION OF PHENYLBORONIC ACID WITH POLYOLS

THE FORMATION OF 5- or 6-MEMBERED CYCLIC PHENYLBORONATE RINGS, ON THE REACTION OF PHENYLBORONIC ACID WITH POLYOLS

It has been shown that when galactitol reacts with phenylboronic acid, in aqueous methanol, two 6-membered rings (containing 3C, 20 and 1B atom) are formed.

It is known (p.22[13]) that when aldehydes react with polyols, the most favourable arrangement of hydroxyl groups for reaction, is the βC arrangement. If this is not available, the β-arrangement is the next most favoured, followed by the α, αT, βT or γT.

The formation of two cyclic phenylboronate esters, involving two pairs of hydroxyl groups in a β-arrangement, by the reaction of phenylboronic acid with galactitol, is in agreement with these rules for acetal formation. There are no hydroxyl groups in the βC arrangement in galactitol.

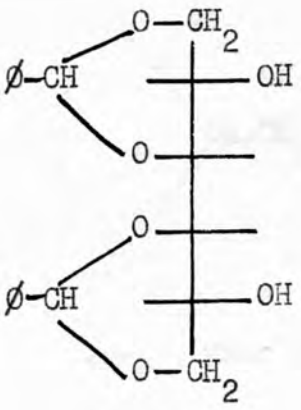
It is of interest to consider why 6-membered rings are formed in the case of galactitol and a 5-membered ring in the case of glycerol.

There is not, in general, a definite agreement between the reactions of hexitols and glycerol with a particular reagent.

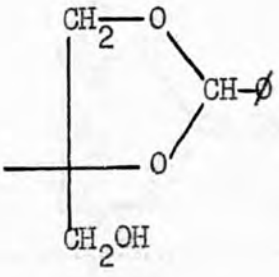
The reaction between benzaldehyde, and galactitol and

glycerol, can be considered as an example.

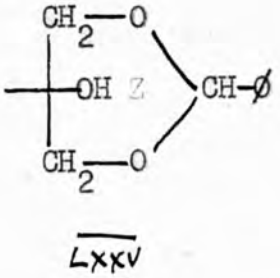
The reaction of benzaldehyde and galactitol produces 1,3:4,6-di-O-benzylidene-galactitol (XLIX). Two pairs of hydroxyl groups in the β-arrangement react. However, when glycerol and benzaldehyde react together, a mixture of 1,2-mono-O-benzylidene-glycerol (LXXIV) and 1,3-mono-O-benzylidene-glycerol (LXXV) is obtained, always with an excess of the 5-membered ring. The actual ratio of the products varies from 7.5 parts of 5-membered ring to 1 part of 6-membered ring when glycerol and benzaldehyde are heated together at a temperature $> 145^{\circ}C$ in an atmosphere of CO_2, to 3 parts of 5-membered ring to 1 part of 6-membered ring, when benzaldehyde and glycerol are heated together, with a little conc. H_2SO_4. The two products can be separated, and if a pure specimen of either is treated with dry HCl, the final ratio of the quantity of 5-membered to 6-membered rings is approximately 5:1.



XLIX



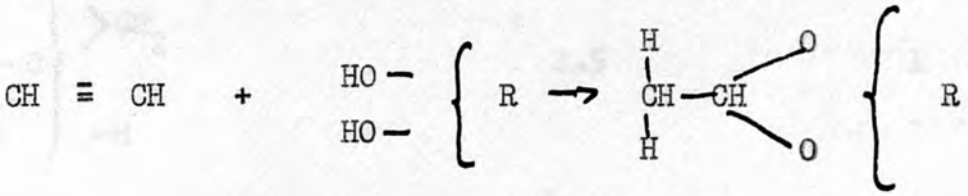
LXXIV



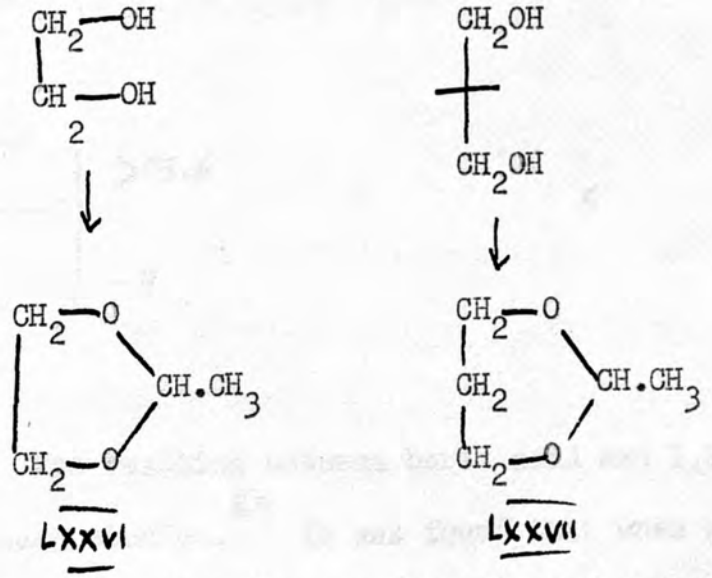
LXXV

Reactions between glycerol and other compounds give products with varying ring sizes, dependent on the nature of the reacting compound.

By the reaction of acetylene with diols [46] it has been shown that a 6-membered ring is preferable to a 5-membered ring. The reaction of acetylene with a glycol produces an acetal, which is apparently derived from acetaldehyde and the glycol.



In a reaction in which ethylene glycol and 1,3-propane-diol complete for acetylene, the ratio of the compound (LXXVI):(LXXVII) is 2:1.

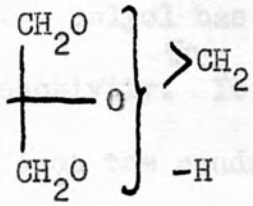
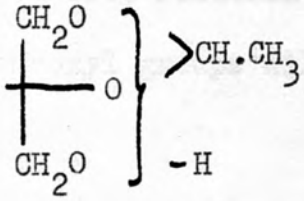
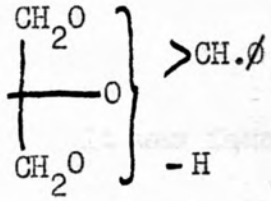


LXXVI

LXXVII

However, when the proportions of 5- and 6-membered rings are considered in reactions of glycerol and other compounds, it is found that 5-membered rings are preferentially formed.

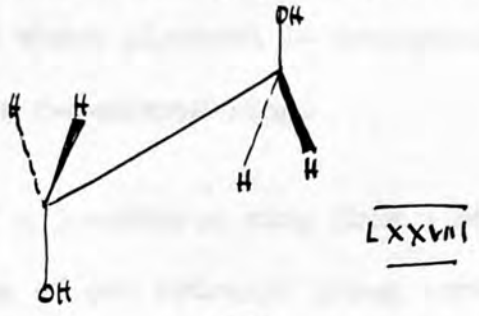
The following table shows the varying proportions of 5- and 6-membered rings depending on the size of the reacting group.

	Proportions of ring sizes	
	5-membered	6-membered
	1.5	1
	4-1.8	1
	5	1

The reaction between boric acid and 1,2- and 1,3-diols has been studied.[49] It was found that when a mixture of 1 mol. ethylene glycol and 1 mol. propane-1,3-diol, was treated with 1 mol.

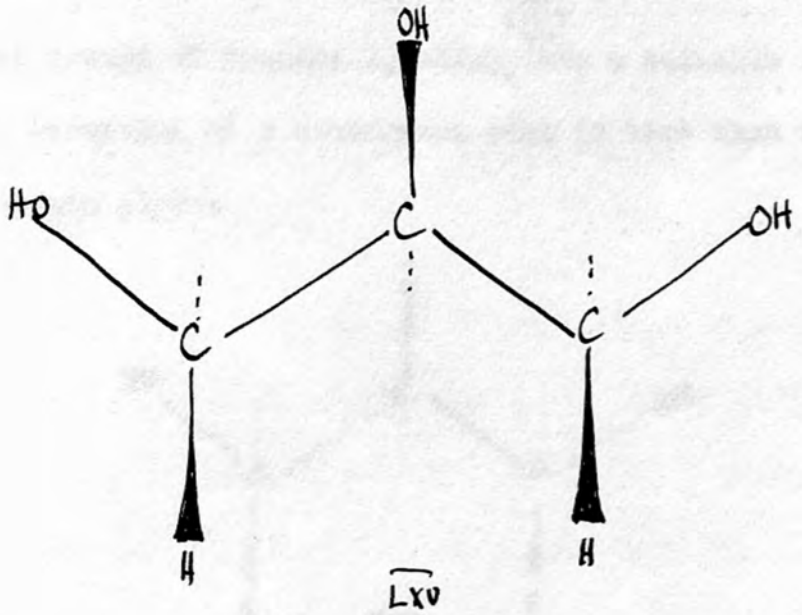
boric acid, most of the boric acid reacted with the 1,3-diol. It is suggested that a 5-membered ring is strained, when boron is trivalent, but not with tetravalent boron; and that a 6-membered ring is not strained with either tri- or tetravalent boron.

In considering the reaction of boric acid with polyols, the change in conductivity of boric acid solution on the addition of a polyol has been used as a criterion for estimating the reactivity.[50] It was found that ethylene glycol itself did not affect the conductivity of boric acid. This is because the position of the hydroxyl group in (LXXVII) is not favourable for reaction. With free rotation of the hydroxyl groups about the C-C bond, the hydroxyl groups will move as far apart as possible.



LXXVIII

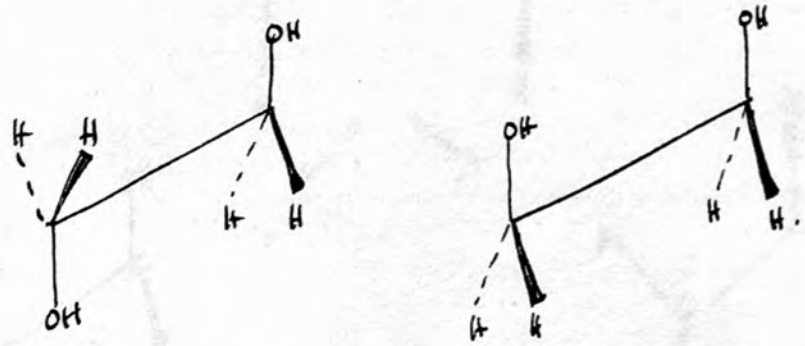
It was found that glycerol (LXV) in which the third hydroxyl group prevents those on carbons 1 and 2 from being as widely separated as in ethylene glycol (LXXVII), does cause an increase in the conductivity of boric acid solution, and that other polyols with more than three hydroxyl groups increase this effect.



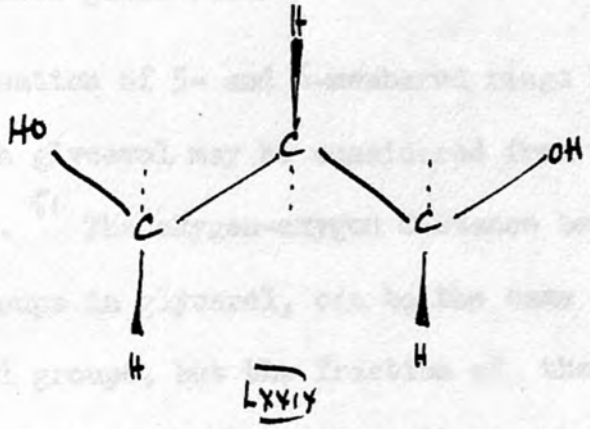
LXV

Thus, in considering the reactions of carbonyl compounds and boric acid with diols and with glycerol, it is apparent that where diols are concerned a 6-membered ring is preferable to a 5-membered ring, and where glycerol is concerned a 5-membered ring is preferable to a 6-membered ring.

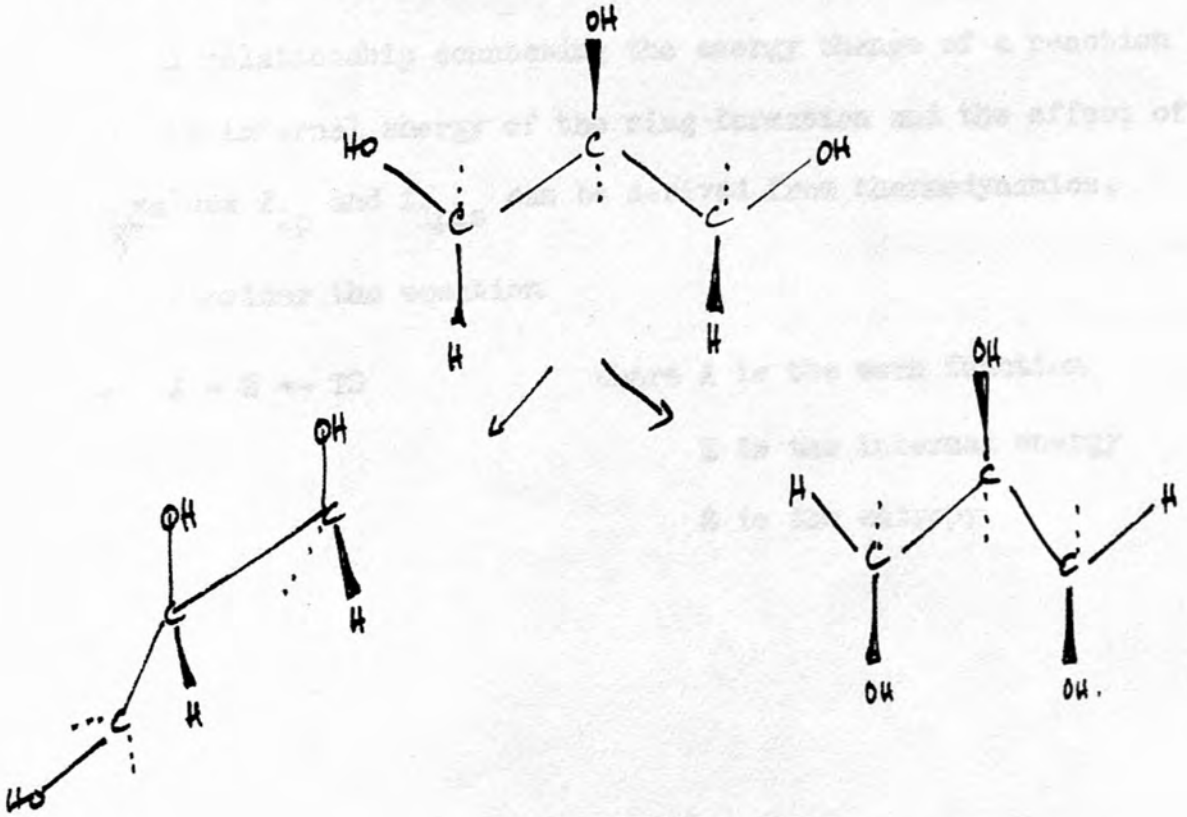
The formation of a 5-membered ring from a ethylene glycol (LXXVIII) requires rotation of one hydroxyl group through a large angle



The rotation of hydroxyl groups required to bring the hydroxyl groups of propane-1,3-diol into a suitable arrangement for the formation of a 6-membered ring is less than that necessary for ethylene glycol.



In the case of glycerol, the rotation necessary to bring the 1 and 2 hydroxyl groups into a suitable arrangement for reaction is less than that required to bring the 1 and 3 hydroxyl groups into a similar arrangement.



Thus the formation of a 5-membered phenylboronate ring by the reaction of phenylboronic acid with glycerol is consistent with this evidence, although in contrast to the reaction of phenylboronic acid with galactitol.

The formation of 5- and 6-membered rings by reaction of compounds with glycerol may be considered from a thermodynamic point of view.[51] The oxygen-oxygen distance between the 1 and 3 hydroxyl groups in glycerol, can be the same as that of the 1 and 2 hydroxyl groups, but the fraction of the glycerol molecules, f_{2p} with this 1,3 oxygen distance small enough for reaction with another compound, will be less than the fraction f_{1pls} of the molecules with the 1-2 oxygen-oxygen distance suitable for reaction. (Where p and s denote primary and secondary -OH groups, respectively).

A relationship connecting the energy change of a reaction with the internal energy of the ring formation and the effect of the values f_{2p} and f_{1pls} can be derived from thermodynamics.

Consider the equation

(a) $A = E - TS$ where A is the work function

E is the internal energy

S is the entropy

Using the statistical formula

(b) $S = \frac{E}{T} + k \ln Z$ where E is the internal energy

Z is the molar partition function

$= f^N$ where f is the molecular partition function

S is entropy

$$ST = E - RT \ln \frac{1}{f}$$

Substituting in (a) at equilibrium when the energy change = 0

$$0 = -A + RT \ln \frac{1}{f}$$

Thus for the formation of a cyclic phenylboronate from glycerol and phenylboronic acid, two equations can be written

$$0 = -A_5 + RT \ln \frac{1}{f_{1pls}}$$

$$0 = -A_6 + RT \ln \frac{1}{f_{2p}}$$

A_5 = energy for formation of a 5-membered cyclic ester

A_6 = energy for formation of a 6-membered cyclic ester

The value of the term f_{1pls} will be greater than the value of f_{2p}. Therefore the term $RT \ln \frac{1}{f}_{1pls}$ will be less than the term $RT \ln \frac{1}{f_{2p}}$.

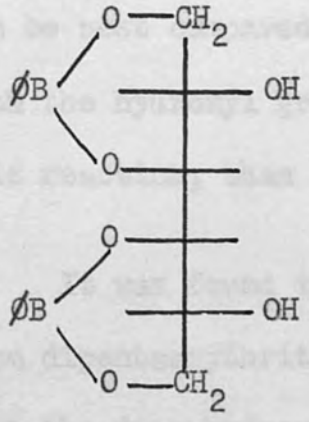
These two terms will have values which will be fairly constant for reactions of glycerol, in which the stereochemistry of the reacting groups are similar.

Thus the proportion of 5- and 6-membered rings formed will be dependent on the difference between the RT terms and the difference between the terms A_5 and A_6.

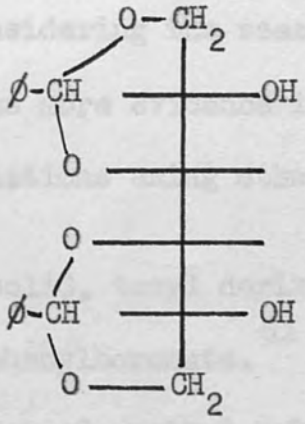
DIFFERENCES IN THE REACTIVITY OF THE FREE HYDROXYL GROUPS OF PHENYLBORONATES, AND DISCUSSION ON THE CONFORMATIONAL ARRANGEMENTS OF PHENYLBORONATES

Differences in the reactivity of the free hydroxyl groups of phenylboronates, and discussion on the conformational arrangements of phenylboronates

During reactions carried out to determine the structure of galactitol bisphenylboronate (XXVI) it was found that the free hydroxyl groups in this compound were much more difficult to substitute, than those in 1,3:4,6-di-O-benzylidene -galactitol (XLIV)



XXVI

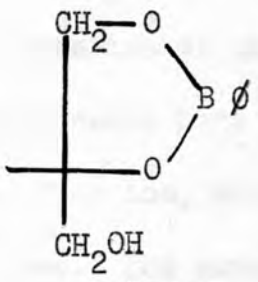


XLIV

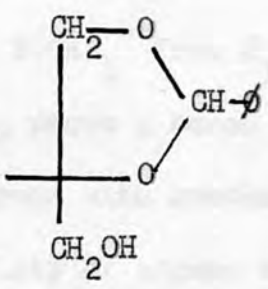
The hydroxyl groups in these two compounds appear to be similarly placed, in relation to the rest of the molecule, and the great difference in their reactivity seems surprising.

Similarly, the reactivity of the free hydroxyl group in

glycerol monophenylboronate (XXIX) is much less than a similar hydroxyl group in e.g. benzylidene glycerol (LXXIV).



XXIX

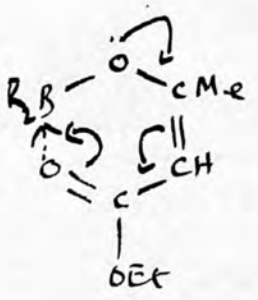


LXXIV

The reactivities of the free hydroxyl groups in phenylboronates can be best compared by considering the reaction of tosyl chloride with the hydroxyl groups, as more evidence is available for this reaction, than for reactions using other reagents.

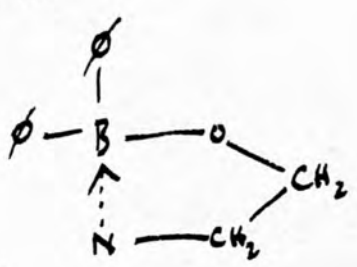
It was found that a solid, tosyl derivative can be easily prepared from dipentaerythritol-bisphenylboronate.[52] Ferrier[5] reported that the free hydroxyl groups of methyl α-D-glucopyranoside-4,6-phenylboronate (XII) reacted with tosyl chloride, although a crystalline product was not isolated. There was no reaction between tosyl chloride and the 1,3:4,6-bisphenylboronate of galactitol (XXVI), although the hydroxyl groups on the 2 and 5 positions of 1,3:4,6-di-O-benzylidene galactitol (LXIX) can be tosylated easily.[24] Glycerol-1,2-monophenylboronate (XXIX) was also unreactive towards tosyl chloride.

It seems likely that the conformations of the various phenylboronate rings are responsible for the variation in reactivity of the hydroxyl groups concerned. In conjunction with this, is the tendency of the boron atom to acquire electrons, as in the formation of the borate ion $B(OH)_4^-$ from H_3BO_3 and OH^-.[53] Certain cases have been reported, where a boron atom, while not forming an ion, shares two electrons with another atom or group of atoms. The exceptional stability of organo boron ethyl acetoacetates to atmospheric oxygen and hydrolysis has been attributed to the formation of a chelate and to the apparent aromatic character of the ring (LXXX).[54]



LXXX

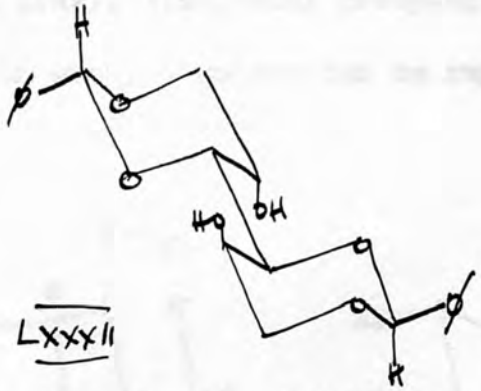
Similarly, β-aminoethyl, diphenylborinate, (LXXXI) which was shown to be monomeric, was found to be very stable to water, and could be stored for long periods without decomposition.[55] Its structure was proposed as



LXXXI

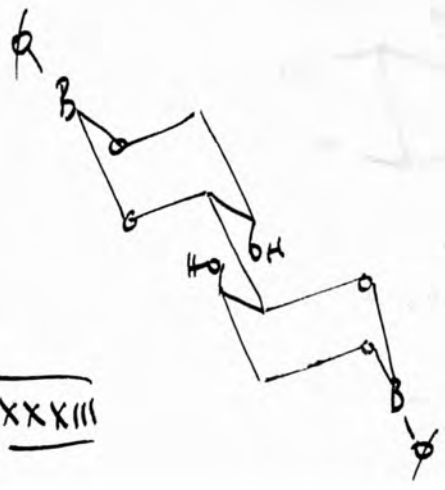
It is thought that it is this type of co-ordination by the boron atom, which affects the conformations of some phenylboronates, and reduces the reactivities of the hydroxyl groups.

1,3:4,6-di-O-Benzylidene-galactitol which can be represented as in (LXXXII), can be easily converted to 2,5-di-O-tosyl-1,3:4,6-di-O-benzylidene-galactitol.[24]

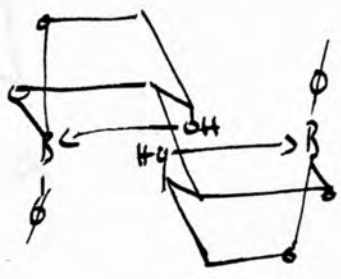


LXXXII

A similar structure for the 1,3:4,6-bisphenylboronate of galactitol, is that given by (LXXXIII). This compound cannot be tosylated. However, if the conformation of (XXVI) is that given by (LXXXIV), then, the interaction of the hydroxyl groups with the boron atoms, could prevent their substitution by the tosyl group.



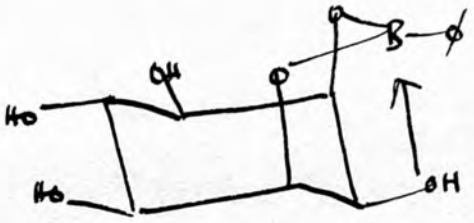
LXXXIII



LXXXIV

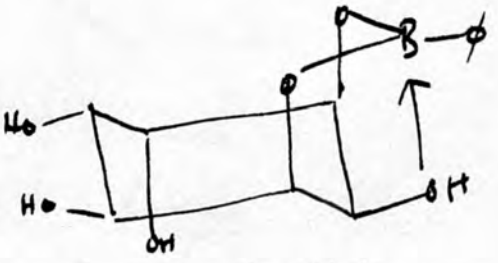
It is interesting to consider the structure (LXXXIV) when the effect of the addition of phenylboronic acid to an acidic chromatography solvent on the R_F values of polyols is studied.

The R_F values of epi-inositol and allo-inositol are greater in a solvent containing phenylboronic acid, than in the solvent from which the phenylboronic acid was omitted. Both these compounds contain a 1(ax), 3(ax)-diol grouping with a 2(eq)-hydroxyl group. Reaction with phenylboronate can be represented by structures (LXXXV) and (LXXXVI).



epi-inositol

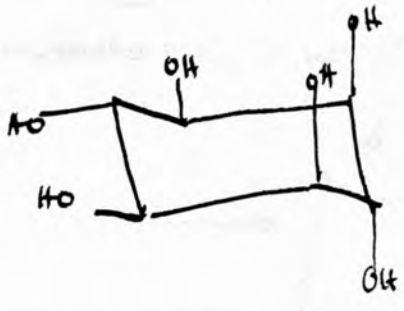
LXXXV



allo-inositol

LXXXVI

This type of structure is not possible with muco-inositol, (LXXXVII) and its R_F value is unaffected by the presence of phenylboronic acid.

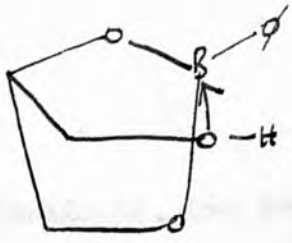


muco-inositol

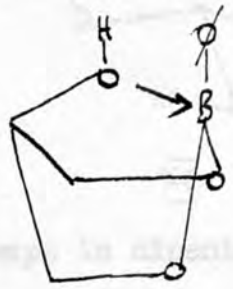
LXXXVII

The "tridentate" structure of the phenylboronate, with interaction between the free hydroxyl group, and the boron atom, which is shown in structures (LXXXVII) and (LXXXVII) is the same as the arrangement of the two6-membered rings in the 1,3:4,6-bisphenylboronate of galactitol (LXXXIV).

The low reactivity of the hydroxyl group in glycerol monophenylboronate may be explained by a similar type of structure (LXXXVII).



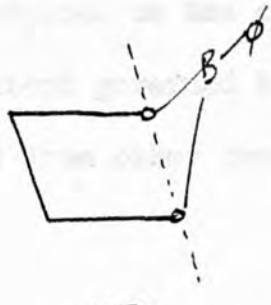
LXXXVIII



LXXXIX

The production of only a 1-substituted glycerol monourethane from glycerol monophenylboronate, indicates that this compound is in the form, (LXXXVII) and is not a mixture of (LXXXVIII) and (LXXXIX) - which would give a mixture of products on reaction with phenyl isocyanate.

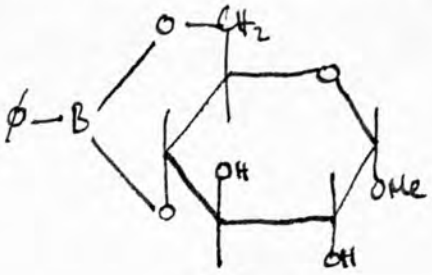
This structure (LXXXVIII) is stereochemically possible, if the 5-membered phenylboronate ring is not planar, but is bent about the O-O axis (XC)



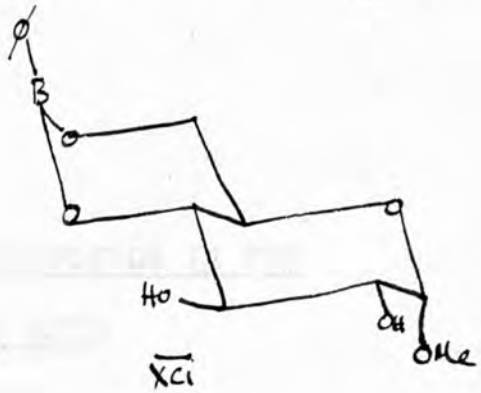
XC

5

It has been shown that the free hydroxyl groups of methyl α-D-glucopyranoside-4,6-phenylboronate (XII) react with tosyl chloride. The hydroxyl groups in this compound are not in a suitable position for the formation of a "tridentate" phenylboronate structure, with boron-oxygen interaction (XCI).

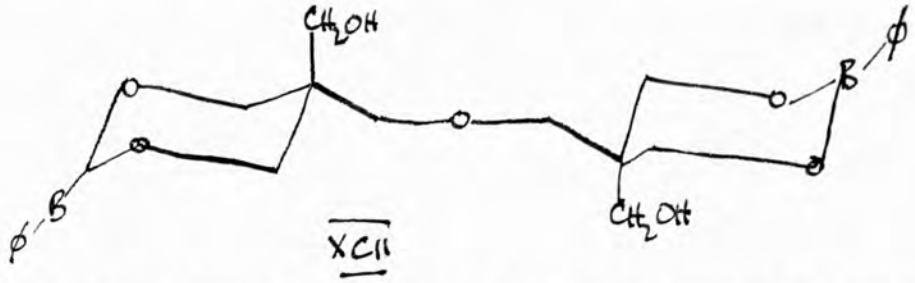


XII



XCI

Similarly, the free hydroxyl groups in dipentaerythritol bisphenylboronate (XCII) are not suitably arranged for the formation of a "tridentate" structure.



XCII

Thus it seems that the reactivity of the hydroxyl groups of phenylboronates depends on the conformations of the phenylboronates, which are to some extent governed by the tendency of the boron atom to acquire electrons from other groups, in this case, the free hydroxyl groups.

THE CHROMATOGRAPHY OF POLYHYDROXY-COMPOUNDS IN THE PRESENCE OF PHENYLBORONIC ACID

THE CHROMATOGRAPHY OF POLYHYDROXY-COMPOUNDS IN THE PRESENCE OF PHENYLBORONIC ACID

Chromatography has been described as "an analytical method for the purification and separation of organic and inorganic substances."[56] It is especially useful for the separation of the components of complex mixtures, and mixtures of closely related compounds. In 1950 Martin wrote that "The essence of the chromatogram is the uniform percolation of a fluid through a column of a more or less finely divided substance, which selectively retards, by whatever means, certain components of the fluid."[57] This definition is a very general one and covers adsorption chromatography, ion exchange chromatography and partition chromatography, which are dependant on very different physico-chemical phenomena.[56] The distinction between these types of chromatography is not always clear. In certain cases separations are achieved when it is not known which phenomenon is concerned or when a combination of two types of chromatography is responsible for the effect.

Adsorption Chromatography

The classical form of chromatography was that which is

now described as adsorption chromatography. In its earliest form substances were separated by filtering their solutions through a finely powdered adsorbant in a glass tube and then washing ("developing") the column of adsorbant with a solvent.[58] The substances which were first separated by this method were coloured compounds and could therefore be seen on the column. When separation was sufficient the adsorbant was extruded from the tube as a column and the compounds removed from separate parts of the adsorbant by suitable solvents. This original technique has been superseded by the 'liquid' or 'flowing' chromatogram of Reichstein[59] and others. In this method the column is washed with a series of solvents of stronger and stronger eluting power, each filtrate being collected separately. A further development in this method is known as gradient elution. The composition of the eluting solvent is changed gradually and compounds are removed from the column with better separation than with the flowing chromatogram method.

Ion Exchange Chromatography

In contrast to the field of adsorption chromatography where the original techniques were developed using organic materials, fundamental work on ion exchange chromatography

was carried out in connection with problems in inorganic chemistry.[60] Ion exchange resins are used in many industrial processes. In conjunction with the technique of chromatographic elution they can be used for a variety of separations in inorganic and organic chemistry. Many synthetic resins have been prepared.[61] They contain either basic or acidic groups which will always be occupied by ions of the opposite charge. An acidic resin holding H^+ ions is said to be in the "hydrogen form". If a solution containing sodium chloride is passed through a column of such a resin the Na^+ ions will be removed from the solution and replaced by H^+ ions. Separations of mixtures of ionised substances such as amino acids and nucleotides have been achieved. In the case of amino acids molecular adsorption also occurs and this is a contributary factor in the separation. Separations of polyhydroxy-compounds have been obtained by using a strong base anion exchange resin and a borate buffer of pH 8-9.[62] The formation of a complex with a polyhydroxy-compound causes a decrease in the flow rate of the compound.

Partition Chromatography

"When a solution of a substance is shaken with an immiscible solvent the solute will distribute itself between

two phases and when equilibrium is reached, the coefficient

$$\frac{\text{concentration in solvent A}}{\text{concentration in solvent B}}$$

is a constant α, where α is termed the partition coefficient."[63] In separating a mixture of amino acids Martin and Synge[64] used the differences of partition coefficients of the amino acids with a battery of solvent-solvent extractors. This was developed into the Craig counter-current method of separation but Martin and Synge[65] found that a more efficient method of solvent-solvent extraction is possible. They used a column of silica gel holding 50% water, placed a solution of the mixture on the column and developed the column with a water-immiscible solvent. The liquid on the column is known as the stationary phase and the eluant is known as the mobile phase. This process is called partition chromatography. Consden, Gordon and Martin[66] showed that filter paper sheets or strips can be used as a support for the stationary phase in partition chromatography. This technique is called paper chromatography and is one of the most valuable techniques for analysis on a micro scale. Water is the most common solvent used as the stationary phase and less polar organic

solvents are used as mobile phases. The relative solubility of a compound in water and organic solvents affects the rate of movement of the compound on a chromatogram. Compounds more soluble in water move slowly as water is the stationary phase, whereas those more soluble in organic solvents (the mobile phase) move faster. The rate of movement of a compound on a paper chromatogram is usually given by its R_F value.

The R_F value of a compound is defined as

$$\frac{\text{the distance moved by the compound}}{\text{the distance moved by the solvent front in the same time}}$$

In some cases it is desirable to express the rate of movement of a compound relative to that of a reference compound, e.g. glucose.

The R_G ($R_{Glucose}$) value of a compound is defined as

$$\frac{\text{the distance moved by the compound}}{\text{the distance moved by glucose in the same time}}$$

Paper partition chromatography was first applied to monosaccharides by Partridge [67] in 1946. An indication of the wide variety of solvents used in the carbohydrate field is given by Bailey and Pridham [68] who state that some 60 of the

reported solvents for sugars appear to be of value for oligosaccharide separations.

Electrophoresis

Electrophoresis involves the migration of charged substances in a conducting solution under the influence of an applied electric field. A number of polyols react with certain inorganic ions to give ionic complexes. Thus, in electrolytes containing certain inorganic ions, formally neutral polyols can migrate as cations or anions depending on the nature of the electrolyte. In paper electrophoresis the electrolyte is supported by filter paper.

Chromatography in the presence of a reagent which reacts with polyhydroxy-compounds

The various kinds of chromatography have sometimes been carried out in the presence of reagents which react with polyhydroxy-compounds. Derivatives of polyhydroxy-compounds will have different properties from the parent polyol. Thus they may be expected to behave differently from the parent polyol under similar conditions of partition chromatography.

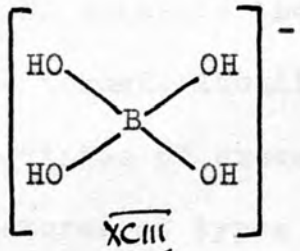
If one compound has a greater affinity for a certain reagent than a second compound it is possible that its partition coefficient in a chromatographic solvent containing

this reagent will be altered enough to cause its separation from the second compound. It is often possible to correlate the affinity of certain polyhydroxy-compounds for reagents with the structures of the polyols. The stereochemical arrangement of the hydroxyl groups can govern the reactions of polyols with certain reagents. For instance, in a reaction of the following type

where $R(OH)_2$ is a polyol and $X(OH)_2$ a reagent such as an inorganic oxy-acid, the structures of both reactants will together decide their point of attachment. Under specified conditions the increase in hydrogen ion concentration of a solution of boric acid on the addition of a polyol can be correlated with the stereochemical arrangement of the hydroxyl groups of the polyol.[50] Paper electrophoresis of formally neutral polyols in electrolytes containing a variety of inorganic ions has already been used to detect certain structural features in unknown compounds.[69]

Boric acid and phenylboronic acid, and their reactions with polyols

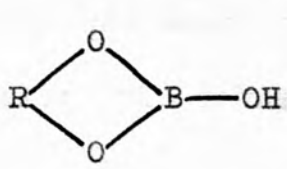
Boric acid is a very weak acid. Its chemical formula is $B(OH)_3$ and it has a dissociation constant of 6.4×10^{-10} at 25°C.[20] It has a planar structure and its oxygen atoms are separated by a distance of 2.36-2.39 Å.[70] In alkaline solution boric acid acts as a Lewis acid[53] and not as a proton donor. It accepts an electron pair from a base e.g. OH^- to form an anion $B(OH)_4^-$ (XCII), in which the B—O bonds are arranged in a tetrahedral manner, and in which the oxygen-oxygen distance is 2.40-2.44 Å.[70]



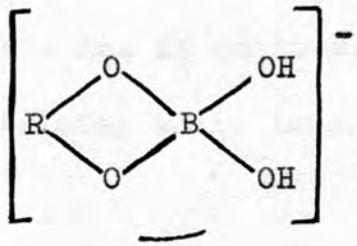
XCIII

Phenylboronic acid is three times as strong an acid as boric acid.[71] The three bonds attaching the hydroxyl groups and the phenyl group to the boron atom are in one plane.

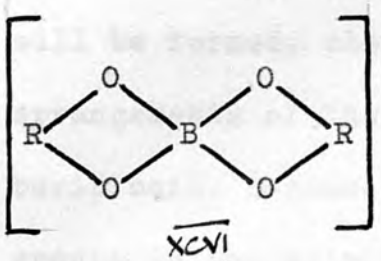
The interactions of boric acid and the borate ion (XCII) with polyhydroxy-compounds have been fully studied. Structures of the following kinds can be produced.[72]



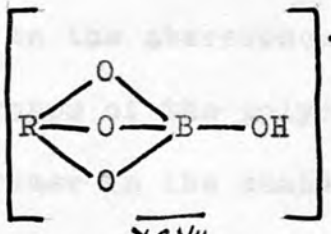
XCIV



XCV



XCVI

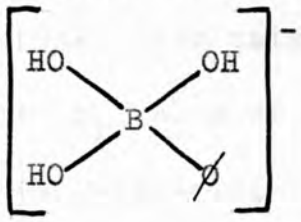


XCVII

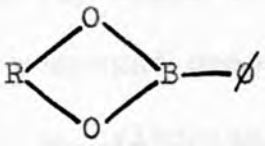
Isbell[72] suggests that in a solution with a high concentration of polyol and a lower concentration of boric acid a complex of type (XCVI) will be most likely, whereas in a solution with a low concentration of polyol and a higher concentration of boric acid a structure of type (XCV) will be more probable. He suggests that in dilute solutions an ester (XCIV) will be formed. Isbell also states that an increase in pH i.e. the provision of excess OH^- ions, will favour the formation of structures of types (XCV) and (XCVI). In an alkaline chromatography solvent incorporating the borate ion complexes of types (XCV), (XCVI) and (XCVII) will be formed from compounds with a suitable stereochemical arrangement of hydroxyl groups. As these complexes are ionic they will be more soluble in water, the stationary phase, and will therefore have a reduced R_F value compared with that in the solvent from which the borate ion is omitted. In an acidic chromatography solvent containing boric acid, neutral esters

will be formed, also depending on the stereochemical arrangements of the hydroxyl groups of the polyol and the boric acid. Because of the decrease in the number of hydroxyl groups in the molecule such a compound will be more soluble in the organic mobile phase, and should therefore have a greater R_F value than in the solvent from which boric acid has been omitted. This is confirmed by Bourne, Hartigan and Weigel[18] who found that in n-Butanol-pyridine-water-sat.aq. boric acid (6:4:2:1 v/v) the R_G value of sorbitol is 0.3. In the solvent from which the borate ion had been omitted the two compounds have the same R_F value. The R_G value of sorbitol in Ethyl acetate-acetic acid-sat.aq.boric acid (9:1:1 v/v) is 2.2 whereas in the solvent from which boric acid is omitted the two compounds again have the same R_F value.

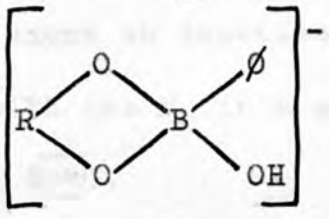
By analogy with the previous discussion on the interaction of boric acid and borate ions with polyols it would be expected that structures of types (XCIX) and (C) will be formed from the interaction of polyols and phenylboronic acid or its phenylboronate ion (XCVIII).



XCVIII



XCIX



C

Neutral esters (XCIX) will probably be formed in neutral and acid solutions. Incorporation of phenylboronic acid into an acid or neutral chromatography solvent should cause an increase in the R_F value on ester formation. The presence of a phenyl group in the neutral ester should make it considerably more soluble in the organic phase than the parent polyol. An alkaline solvent, which probably contains the phenylboronate ion (XCVIII) should result in the formation of a complex of type (C). The effect of this on the R_F value of a compound, compared with the R_F in the solvent without phenylboronic acid will be two-fold. The ionisation of the complex will cause it to be more soluble in water than the parent polyol and this will tend to reduce the R_F value in comparison with that in the solvent from which the phenylboronate ion (XCVIII) has been omitted. This effect is the same as that with a solvent containing the borate ion (XCIII). However, in the case of a complex of type (C) the presence of an aromatic group will oppose the effect of the ionisation and will tend to make the complex more soluble in the organic phase. Thus this group could tend to cause an increase in the R_F value of a compound compared with the R_F in a solvent not containing the phenylboronate ion (XCVIII).

It seems possible that the incorporation of phenylboronic acid or the phenylboronate ion into chromatography solvents could cause separations of polyols by the preferential reaction of the acid or ion with certain stereochemically suitable arrangements of hydroxyl groups.

The anionic complexes produced from borate ions and polyhydroxy-compounds form a basis of a method of separating such compounds by electrophoresis. The rate at which a compound moves on electrophoresis depends on the degree of ionisation of the compound or its complex. This is related to the strength of the complex which in turn depends on the stereochemistry of the polyol in relation to the borate ion. Foster [73] has described this method of studying polyol structure. If phenylboronic acid is converted in the presence of alkali to the phenylboronate ion (XCVIII) then electrophoresis using a phenylboronate electrolyte should be possible.

The R_F values of certain polyhydroxy-compounds were determined using acidic, basic and neutral chromatography solvents. In all cases the R_F values were compared with those in the appropriate solvent from which phenylboronic acid had been omitted. (Expts. 41)

The result of the presence of the phenylboronate ion (XCVIII)

in a chromatography solvent was found to be different from the effect of a borate ion in the same solvent. As previously reported, the R_G value of sorbitol in n-Butanol-pyridine-water-sat.aq.boric acid (6:4:2:1 v/v) is 0.3.[18] It was found that the presence of the phenylboronate ion (XCVIII) caused a slight increase in the R_F value of sorbitol compared with that in the solvent from which the phenylboronate ion was omitted.

The following results were obtained using solvents

(b) n-Butanol-pyridine-water (6:4:3 v/v)

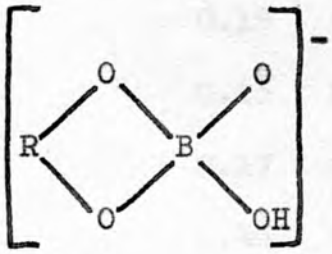
(c) n-Butanol-pyridine-water-sat.aq.phenylboronic acid (6:4:2:1 v/v)

Table I

Compound	R_F in solvent	
	(b)	(c)
Mannitol	0.32	0.38
Sorbitol	0.32	0.38
Dulcitol	0.32	0.38
Glycerol	0.52	0.56
Erythritol	0.45	0.49
Glucose	0.27	0.32

These values show a small but consistent increase in the R_F values of the compounds concerned, in the presence of the phenylboronate ion (XCVIII).

In considering the complex type (C) and the opposition



of the effect of ionisation to that of the presence of a phenyl group, it seems that the effect of the phenyl group is slightly stronger than that of the ionic character of the complex.

The presence of phenylboronic acid in a neutral solvent was also studied, and the R_F values of certain polyols in such a solvent compared with those in the solvent from which phenylboronic acid had been omitted, and with a solvent where the phenylboronic acid had been replaced by boric acid. The following solvents were prepared and the results are listed in Table II.

(d) ethyl acetate-ethanol-water (11:4:2 v/v)

(e) ethyl acetate-ethanol-sat.aq.boric acid (11:4:2 v/v)

(f) ethyl acetate(0.45% phenylboronic acid solution)-ethanol-water (11:4:2 v/v)

Table II

Compound	R_F in solvent		
	(d)	(e)	(f)
Glucose	0.18	0.14	0.17
Mannitol	0.19	0.20	0.30
Sorbitol	0.18	0.23	0.38
Dulcitol	0.17	0.21	0.29
Glycerol	0.40	0.39	0.37
Myo-inositol	0.06	0.04	0.05

The presence of boric acid in the neutral solvent is seen to have a negligable effect on the R_F values of the compounds examined. The presence of an acid is obviously necessary for the formation of esters of type (XCIV), probably because boric acid is such a weak acid. Barker and Smith[74] report that some compounds have a lower R_F value in an n-Butanol-boric acid solution solvent than in an n-Butanol-water solvent. It seems that phenylboronic acid is itself a strong enough acid for neutral ester formation without the presence of another acid.

The formation of a complex of type (C) in the presence of the phenylboronate ion (XCVIII) can be demonstrated by paper

electrophoresis, using a solution of phenylboronic acid in water (1%) adjusted to pH 10 with sodium hydroxide solution (1N). Garegg and Lindberg[75] have used a solution of sulphonated phenylboronic acid in water, at pH 6.5, as an electrolyte. Considerable movement of certain polyols was observed. The effect of the sulphonic acid group on the boronic acid group is to decrease its ionisation, thus leading to trigonal boron. Because of this, the products formed at pH 6.5 are in all likelihood esters of boronic acid, their migration being due to the ionisation of the sulphonic acid group.

The results obtained using a solution containing phenylboronate ions, at pH 10, are listed in the following Table. The values quoted for electrophoresis in borate solution are those given by Frahn and Mills[76].

The M_G value of a compound is defined as

$$\frac{\text{the true distance of migration of the compound}}{\text{the true distance of migration of glucose}}$$

In Table III below 'E' indicates an extended spot.

Table III

Compound	$10^2 \times M_G$ in borate	$10^2 \times M_G$ in phenylboronate
D-Glucose	100	100
D-Galactose	93	70
D-Mannose	69	64
DL-Glycerose	-	55
D-Arabinose	91	71
D-Ribose	75	74
D-Lyxose	71	74
2-deoxy-D-Glucose	-	43
3-O-methyl-D-Glucose	76	76
5-deoxy-D-Glucose	-	80
2-deoxy-D-Allose	-	54
2-deoxy-D-Ribose	-	27
2-deoxy-D-Galactose	-	34
6-deoxy-L-Galactose	83E	82E
6-deoxy-L-Mannose	49	50
α-methyl-D-Glucopyranoside	10	5

Table III cont.

Compound	$10^2 \times M_G$ in borate	$10^2 \times M_G$ in phenylboronate
D-Fructose	89	77
L-Sorbose	97	80
Sucrose	16	10
4-O-α-D-Glucopyranosyl-D-glucose (Maltose)	30	33
6-O-α-D-Glucopyranosyl-D-glucose (Isomaltose)	-	53E
4-O-β-D-Galactopyranosyl-D-glucose (Lactose)	37	30
6-O-β-D-Glucopyranosyl-D-glucose (Gentiobiose)	-	62
6-O-α-D-Galactopyranosyl-D-glucose (Melibiose)	77	63
D-Glucitol	83	76
2-deoxy-	-	75
3-O-methyl-	-	55
D-Mannitol	91	78

Table III cont.

Compound	$10^2 \times M_G$ in borate	$10^2 \times M_G$ in phenylboronate
Galactitol	97	72
1-deoxy-D-	-	77E
Xylitol	79	78
2-deoxy-D-Ribitol	-	23
Erythritol	75	43
Glycerol	49	13
6-O-α-D-Glucopyranosyl-D-glucitol (Isomaltitol)	-	64
6-O-β-D-Glucopyranosyl-D-glucitol (Gentiobiitol)	-	59
Propane-1,2-diol	16	8
Propane-1,3-diol	5	3
Butane-1,4-diol	0	2
Myo-inositol	49E	53E
Scyllo-inositol	0	0

Using the solution of phenylboronate ions movement of most polyols was observed in a similar pattern to that in electrophoresis in borate solution. Most compounds move more slowly in phenylboronate buffer in relation to glucose than in borate buffer. The glucose marker in a borate buffer moves at the rate of 1.43×10^{-4} $cm^2 v^{-1} sec^{-1}$ whereas the glucose marker in the phenylboronate buffer moves at the rate of 1.90×10^{-4} $cm^2 v^{-1} sec^{-1}$. In addition, borate solution of the usual strength (2%) can be used only up to about 3000v. as above this value the current passing through the system exceeds 100mA. Electrophoresis machines now in use cannot be used above 100mA. without risk of overheating and short circuits. The phenylboronate solution (1%) was used at 5000v. when the current was 75mA.

Dilution of the borate electrolyte, which allows the solution to be used at higher voltages without developing too great a current, was not satisfactory. When the electrophoretograms were developed it was found that the compounds being examined had not remained in discrete spots, but were spread into streaks several centimetres long. Thus a phenylboronate buffer may be used instead of a borate buffer to give the same separations in a shorter time.

The greater acidity of phenylboronic acid will tend to make the complexes stronger, and thus, increasing the ionic strength, should tend to increase the rate of movement on electrophoresis. In general in electrophoresis it is found that larger molecules move more slowly, and therefore the presence of a phenyl group in the complex will tend to slow down the rate of movement.

The effect of the presence of phenylboronic acid in an acidic chromatography solvent (Expt. 41) was studied more fully than its effect in neutral solvents, or than the effect of the presence of the phenylboronate ion in basic solvents.

The following solvents were prepared and the results obtained are given in Table IV below.

(g) ethyl acetate-acetic acid-water (9:2:2 v/v)

(h) ethyl acetate (0.55% solution of phenylboronic acid)-acetic acid-water (9:2:2 v/v)

Table IV

Compound	R_F value in solvent (g)	(h)
Ethane-1,2-diol	0.50	0.50
Propane-1,2-diol	0.59	0.60
Propane-1,3-diol	0.49	0.49
Butane-2,3-diol	0.70	0.68
Butane-1,3-diol	0.67	0.68
Butane-1,4-diol	0.56	0.57
Glycerol	0.32	0.35
Erythritol	0.23	0.31
D-Arabitol	0.14	0.50
1-deoxy-	0.45	0.71
5-deoxy-	0.46	0.85
Ribitol	0.14	0.48
2-deoxy-D-	0.32	0.46
Xylitol	0.14	0.45
Allitol	0.17	0.49
D-Altritol	0.16	0.51
1-deoxy-	0.36	0.85
1,6-dideoxy-	0.57	0.97

Table IV cont.

Compound	R_F value in solvent (g)	(h)
Galactitol	0.07	0.47
1-deoxy-D-	0.31	0.68
1,6-dideoxy-	0.58	0.85
D-Glucitol	0.08	0.45
2-deoxy-	0.22	0.60
3-O-methyl-	0.19	0.44
4-O-methyl-	0.30	0.40
D-Mannitol	0.08	0.43
1,6-dideoxy-	0.58	0.96
2-O-methyl-	0.22	0.70
1,2-di-O-methyl-	0.46	0.82
DL-Glycerose	0.38	0.40
D-Erythrose	0.31	0.84
L-Threose	0.31	0.53
D-Arabinose	0.12	0.11
D-Lyxose	0.18	0.18
D-Ribose	0.25	0.50
2-deoxy-	0.40	0.41

Table IV cont.

Compound	R_F value in solvent (g)	(h)
D-Xylose	0.15	0.15
D-Allose		
2-deoxy-	0.38	0.40
D-Altrose		
1,6-anhydro-β-pyranose	0.20	0.19
D-Galactose	0.06	0.08
6-deoxy-	0.19	0.18
1,6-anhydro-β-pyranose	0.33	0.38
D-Glucose	0.08	0.08
3-O-methyl-	0.21	0.23
5-deoxy-	0.28	0.27
methyl α-pyranoside	0.20	0.21
1,6-anhydro-β-pyranose	0.33	0.31
D-Gulose	0.13	0.27
1,6-anhydro-β-pyranose	0.31	0.30
D-Talose	0.10	0.17
L-Idose	0.09	0.16
D-Mannose	0.08	0.09
6-deoxy-	0.22	0.25

Table IV cont.

Compound	R_F value in solvent (g)	(h)
D-Mannose		
3,4-di-O-methyl-	0.55	0.59
methyl α-pyranoside	0.42	0.42
1,6-anhydro-β-pyranose	0.33	0.39
D-Fructose	0.11	0.12
L-Sorbose	0.10	0.16
D-Glucitol		
2-O-α-D-glucopyranosyl- (kojibiitol)	0.02	0.04
β-isomer (sophoritol)	0.02	0.04
3-O-α-D-glucopyranosyl- (nigeritol)	0.02	0.04
β-isomer (laminaribiitol)	0.02	0.04
4-O-α-D-glucopyranosyl- (maltitol)	0.02	0.04
β-isomer (cellobiitol)	0.02	0.05
5-O-α-D-glucopyranosyl- (leucritol)	0.02	0.05

Table IV cont.

Compound	R_F in solvent (g)	(h)
D-Glucitol		
6-O-α-D-glucopyranosyl- (isomaltitol)	0.02	0.16
β-isomer (gentiobiitol)	0.02	0.13
6-O-α-D-galactopyranosyl- (melibiitol)	0.01	0.16
D-Mannitol		
2-O-α-D-glucopyranosyl- (leucritol)	0.02	0.04
Allo-inositol	0.04	0.11
Dextro-inositol		
3-O-methyl	0.09	0.08
Epi-inositol	0.01	0.04
Levo-inositol	0.03	0.02
2-O-methyl	0.07	0.07
Muco-inositol	0.05	0.05
1-deoxy-	0.08	0.08
Myo-inositol	0.02	0.02

Table IV cont.

Compound	R_F value in solvent (g)	(h)
Myo-inositol		
1-deoxy-	0.07	0.06
Scyllo-inositol	0	0

Interpretation of results

There are two hydroxyl groups in phenylboronic acid available for reaction with the hydroxyl groups of a polyol. It is reasonable to assume that these two hydroxyl groups are necessary for reaction (as two are necessary in the reaction between boric acid and a polyol) otherwise there would be no possibility of the steric specificity which is, to a certain extent, apparent in the results.

One would therefore expect that a comparison of the R_F values of a series of diols in solvents (g) and (h) would give some indication of the steric arrangement of hydroxyl groups required for reaction with phenylboronic acid.

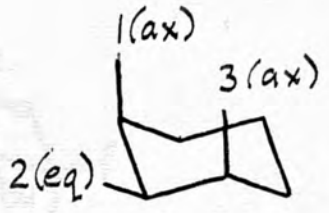
There is evidence to show that a compound which has the same R_F value in solvents (g) and (h) does not necessarily

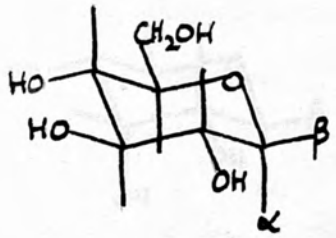
lack a suitable arrangement of hydroxyl groups for reaction with phenylboronic acid. Crystalline phenylboronates of glycerol and glucose have been prepared[77] but the R_F values of these two polyols are not appreciably increased by the presence of phenylboronic acid in an acidic chromatography solvent. It seems likely that under the conditions of the chromatography the equilibrium does not favour the formation of the ester in these two cases.

Certain useful separations can be carried out using the solvent (h). Most of the aldoses and ketoses can be separated from their reduction products in 4-5 hours. The available crystalline phenylboronates are very easily hydrolised by chromatography in a water-containing solvent e.g.solvent (g).[4] Phenylboronic acid has an R_F value slightly less than 1. It can be detected under ultraviolet light, or by the use of the silver nitrate/alcoholic sodium hydroxide solution reagent,[19] when it shows as a pale grey spot. The use of solvent (h) in the separation of e.g. glucose and sorbitol offers an advantage on the preparative scale, over the use of a solvent containing boric acid, in addition to the greatly increased speed of the separation. Boric acid in a solution of a polyhydroxy-compound is removed by

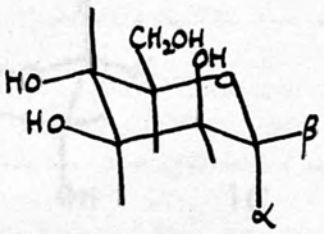
repeated distillation with methanol.[78] This process can result in complications by causing some destruction of the polyol itself. This possibility is avoided when the solution contains phenylboronic acid, as this can be removed from the polyol simply by re-chromatography in solvent (g) or another water-containing solvent such as n-Butanol-ethanol-water (40:11:19 v/v).

Amongst certain classes of compounds some correlation of R_F values with conformations can be seen. The aldoses and cyclitols studied, which have R_F values markedly affected by phenylboronic acid all contain a 1(ax),3(ax)-diol grouping in their more stable conformation. In addition in most cases, the 2-hydroxyl group is in the equatorial position.

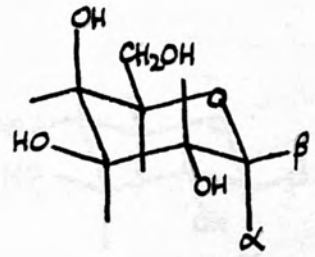




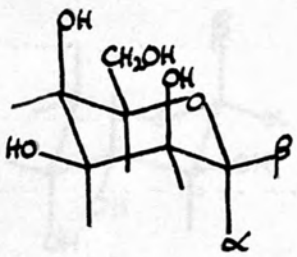
D- Glucose



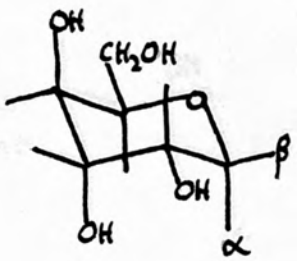
D- Mannose



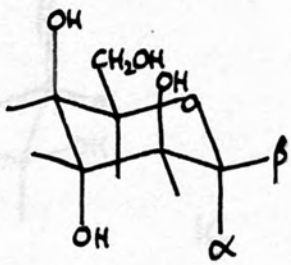
D- Galactose



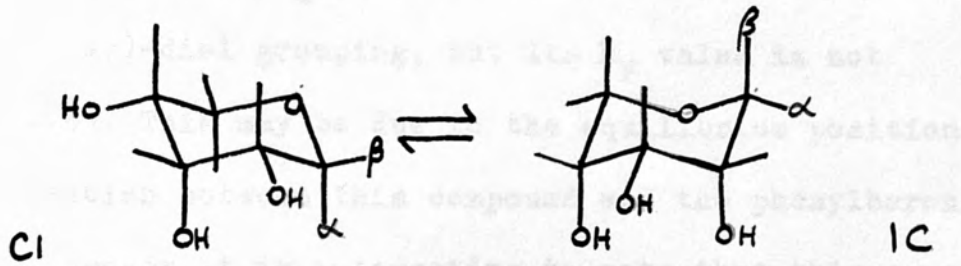
D- Talose



D- Gulose

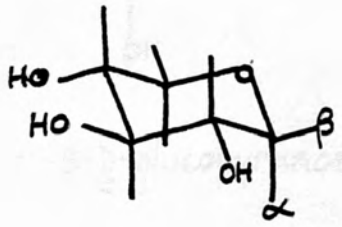


D- Idose

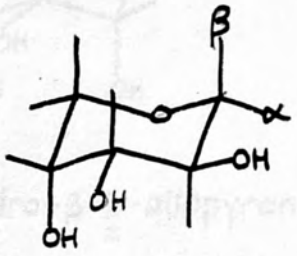


C1 ⇌ 1C

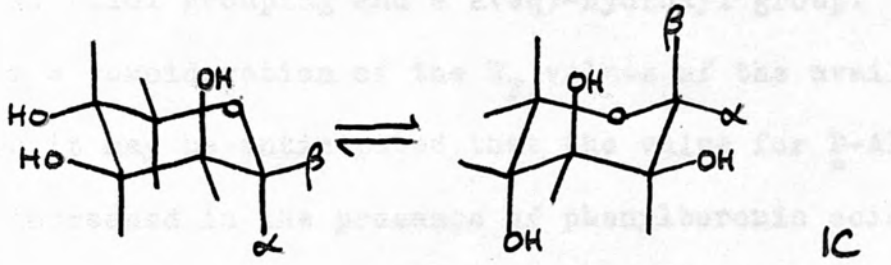
D-Ribose



D-Xylose



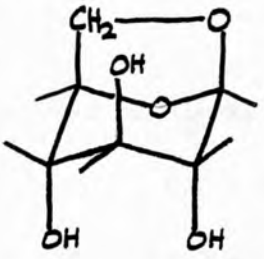
D-Arabinose



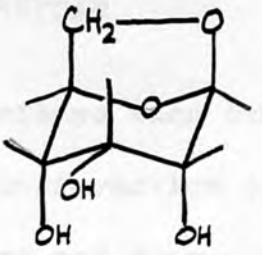
C1 ⇌ 1C

D-Lyxose

1,6-anhydro-β-D-glucopyranoside also contains this 1(ax),3(ax)-diol grouping, but its R_F value is not increased. This may be due to the equilibrium position in the reaction between this compound and the phenylboronic acid. However it is interesting to note that this compound does not contain a 2(eq)-hydroxyl group.



1,6-anhydro-β-D-glucopyranoside

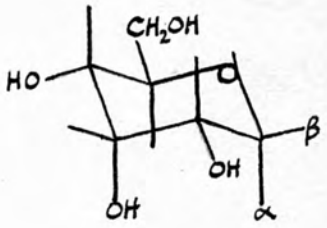


1,6-anhydro-β-D-allopyranoside

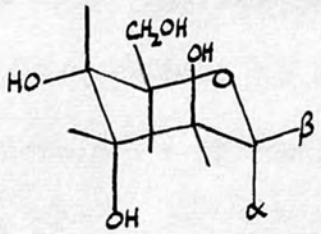
It would be interesting to examine the behaviour of 1,6-anhydro-β-D-allopyranoside, in comparison with that of the glucopyranoside, as the allopyranoside contains a 1(ax),3(ax)-diol grouping and a 2(eq)-hydroxyl group.

From a consideration of the R_F values of the available pyranoses it may be anticipated that the value for D-Allose will be increased in the presence of phenylboronic acid as it contains, in its more stable conformation, a 1(ax),3(ax)-diol grouping, and a 2(eq)-hydroxyl group. The R_F value of D-Altrose may not be increased as it contains only a

1(ax),3(ax)-diol grouping.

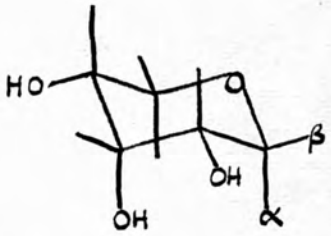


D-Allose

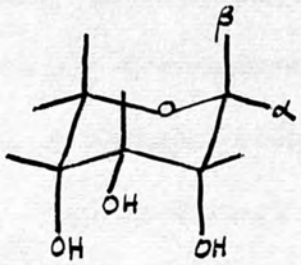


D-Altrose

2-deoxy-D-allose and 2-deoxy-D-ribose each contains a 1(ax),3(ax)-diol grouping in the C1 conformation of its α-anomer. However, their R_F values are not appreciably increased by the presence of phenylboronic acid in the solvent. D-Ribose contains a 1(ax),3(ax)-diol grouping in both its C1 and 1C conformations, in each case with a 2(eq)-hydroxyl group.



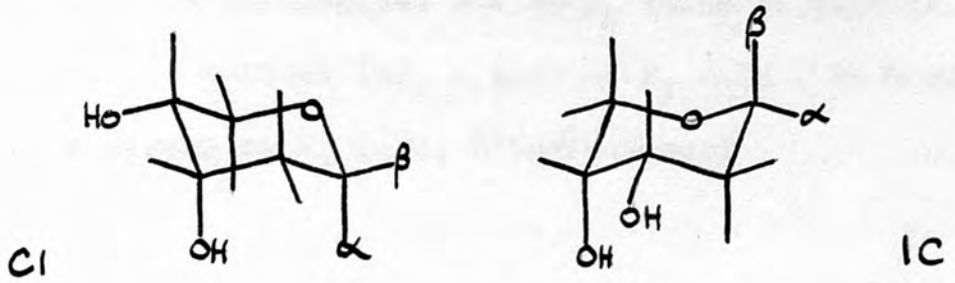
C1



1C

D-Ribose

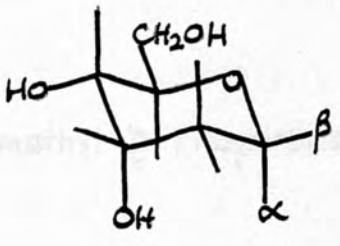
The removal of the 2-hydroxyl group in the C1 conformation of D-Ribose removes the 2(eq)-hydroxyl group, whereas the removal of the 2-hydroxyl group in the 1C conformation removes one of the hydroxyl groups of the 1(ax),3(ax)-diol grouping.



2-deoxy-D-ribose

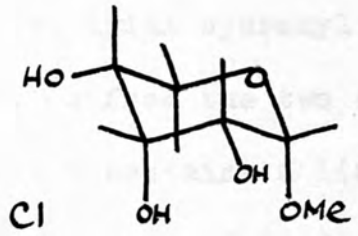
Thus, if in this case three hydroxyl groups are necessary for causing an increased R_F value in the solvent containing phenylboronic acid, then neither conformation of 2-deoxy-D-ribose contains the correct arrangement.

Similarly, 2-deoxy-D-allose, although containing a 1(ax),3(ax)-diol grouping has no 2(eq)-hydroxyl group.

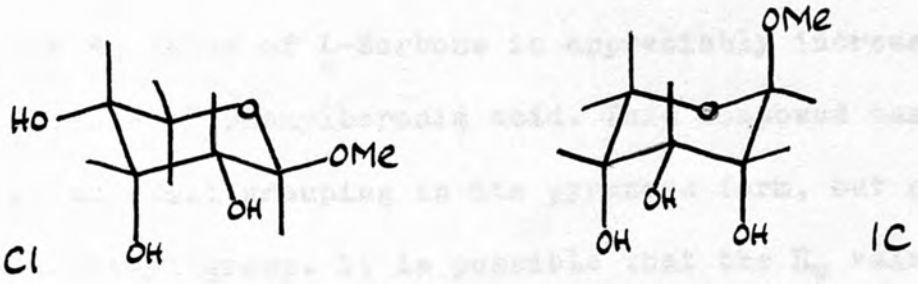


2-deoxy-D-allose

The relationship between the conformations of the α and β-methyl-D-ribopyranosides and their R_F values is not straightforward. β-methyl-D-ribopyranoside has an R_F value of 0.63 in solvent (g). In solvent (h) an elongated spot with an R_F value of 0.80 is found. A mixture of α and β-methyl-D-ribopyranosides has an R_F value of 0.54 in solvent (g), and in solvent (h), a spot of R_F 0.55 (the α-anomer) with a streak to R_F 0.80, (the β-anomer).



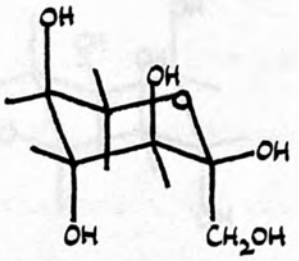
α-methyl-D-ribopyranoside



β-methyl-D-ribopyranoside

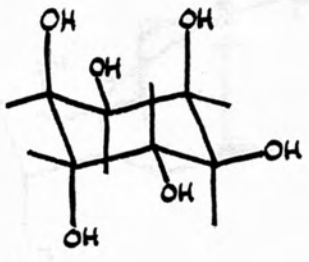
From a study of the behaviour of α-methyl-D-ribopyranoside on electrophoresis in molybdate buffer at pH 5, Angus [79] concluded that this α-anomer was in the C1 conformation. This conformation contains no 1(ax),3(ax)-diol grouping. However, neither does β-methyl-D-ribopyranoside in the C1 conformation. The 1C conformation of the β-anomer would seem unlikely, as it has three axial groups larger than hydrogen, and only one equatorial hydroxyl group. However, the axial -OMe group is on the opposite side of the molecule to the two axial hydroxyl groups, and there will be little interaction from the two axial hydrogen atoms. This arrangement contains a 1(ax),3(ax)-diol grouping and a 2(eq)-hydroxyl group, and it is possible that the increased R_F value of the β-anomer in solvent (h) may be due to this conformation.

The R_F value of L-Sorbose is appreciably increased in the presence of phenylboronic acid. This compound has a 1(ax),3(ax)-diol grouping in its pyranose form, but no 2(eq)-hydroxyl group. It is possible that the R_F value of this compound could be increased by the reaction of phenylboronic acid in position (i),

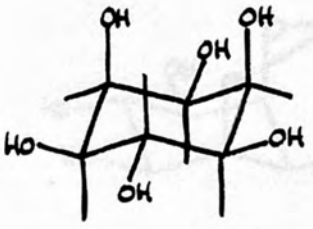


D-Sorbose

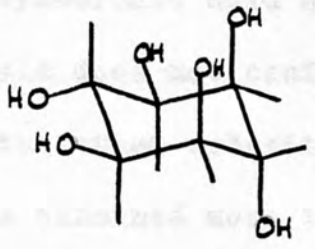
Of the cyclitols studied each of those with an R_F value increased by the presence of phenylboronic acid contains a 1(ax),3(ax)-diol grouping. In addition, muco-inositol, the R_F value of which is not affected, contains this group.



allo-inositol

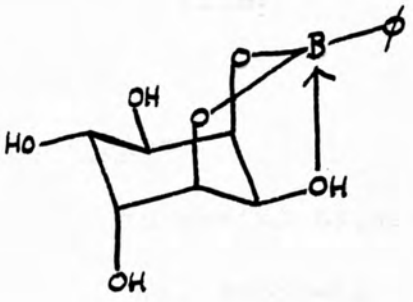


epi-inositol

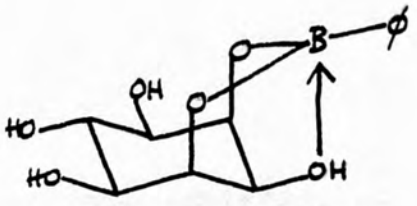


muco-inositol

However, epi- and allo-inositols contain a 2(eq)-hydroxyl group, and reaction with phenylboronic acid can take place as shown below, and including a 6-membered phenylboronate ring. This arrangement is not possible in the case of muco-inositol.



allo-inositol



epi-inositol

The effect of phenylboronic acid on the R_F values of the disaccharide alcohols does not conform to the general pattern of values for the other substituted acyclic polyols. All the acyclic polyols examined move faster in solvent (h) than in solvent (g), and substitution at positions 2,3 or 4 with e.g. a methyl group still results in a considerable increase in R_F value. The only disaccharide alcohols which have an increased R_F value in solvent (h) are those with a (1→6) glycosidic link. This may be due to the size of the substituting group. It is possible that a glucose unit attached to the acyclic polyol could shield the hydroxyl groups available for complexing. Such an effect would be considerably less when the glucose unit was attached to the 6-carbon position.

Conclusion

Certain useful separations have been achieved, in particular the separation of glucose and sorbitol in 4 hours, using a chromatography solvent containing phenylboronic acid.

To a limited extent, correlation of the affinity of the reagent for a polyol is apparent, but the solvent cannot satisfactorily be used as a method for determining the

structures of unknown compounds. This is because the equilibrium position of the reaction between the reagent and a polyol affects the behaviour of the polyol on a chromatogram.

It is possible that separations of e.g. glucose and sorbitol may be practicable on a larger scale, using a charcoal column (as has been used with a solvent containing borate ions[80]) and an eluant containing phenylboronic acid or phenylboronate ions.

ELECTROPHORESIS USING A SOLUTION OF SODIUM STANNATE IN WATER AS AN ELECTROLYTE

ELECTROPHORESIS USING A SOLUTION OF SODIUM STANNATE IN WATER AS AN ELECTROLYTE

Complexes formed between polyhydroxy-compounds and inorganic acids have been used in the separation of mixtures of polyols by electrophoresis.[50]

The elements germanium and tin are both members of Group IV A of the Periodic Table, and for this reason it is of interest to compare the reactions of their oxy-acids with polyols.

The use of an aqueous solution of sodium germanate at pH 10.7 and 40°C as an electrolyte for the paper electrophoresis of polyhydroxy-compounds has been described.[81]

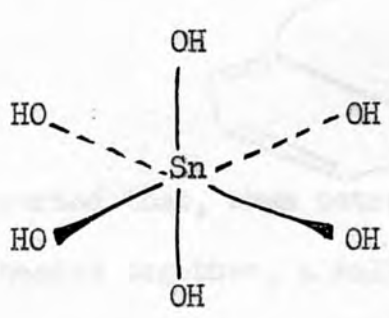
Similarities between the known compounds of germanium and tin, and their reactions, suggest that an aqueous solution of a tin-oxy acid, may be a useful electrolyte for paper electrophoresis.

Germanic acid, in solution, readily undergoes polymerisation reactions, depending on the pH of the solution.

At pH 9, the pentagermanate ion $Ge_5O_{11}^{--}$ is present in solution.[82] An increase or decrease in pH causes depolymerisation giving the ion $HGeO_3^-$ or $Ge(OH)_6^{2-}$, at pH 11. The O-O distances in this

octahedral anion $Ge(OH)_6^{2-}$ have been calculated to be about 2.64 Å.[81] This calculation is based on the Ge-O distance in tetrahedral germanium compounds, which is assumed to be similar to the Ge-O distance in the $Ge(OH)_6^{2-}$ ion, which has not been measured.

Sodium stannate, the structure of which can be represented as $Na_2O.SnO_2.3H_2O$, can be prepared by the treatment of stannic oxide with sodium hydroxide solution.[83] It derives from the octahedral anion $Sn(OH)_6^{2-}$.

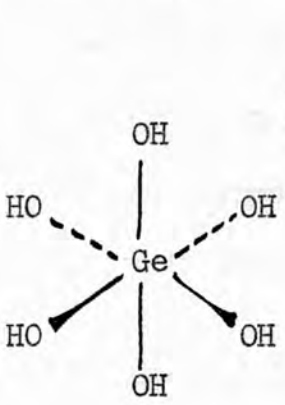


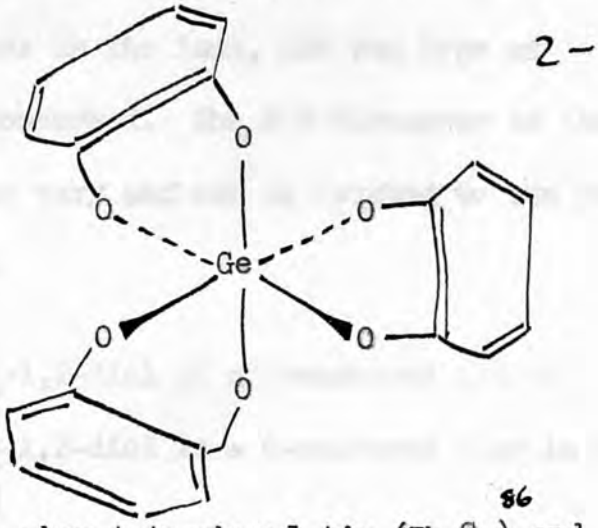
From the dimensions determined for the crystal structures of sodium stannate[84] $Na_2Sn(OH)_6$ and potassium stannate, an average O-O distance of 2.77Å can be calculated for the $Sn(OH)_6^{2-}$ ion.

Reaction has been reported between compounds of both germanium and tin, with phenols. Data obtained in an investigation of

the neutralisation of a solution containing GeO_2 and an o-diphenol,[86] indicates the formation of a germani-diphenolic acid. The suggested structure of such an acid is

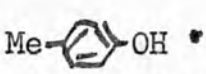
$H_2[Ge(O_2.C_6RR'R''R''')_3]$ which has the same co-ordination number (6) for germanium as has the ion $Ge(OH)_6^{2-}$.





It has been reported that, when tetraphenyl tin (Ph_4Sn)[86] and

are heated together, a solid compound with the structure



$\cdot SnO_3.3H_2O$ is formed. In addition the preparation of a complex salt is described,[87] by reaction between potassium stannate and benzidine hydrochloride

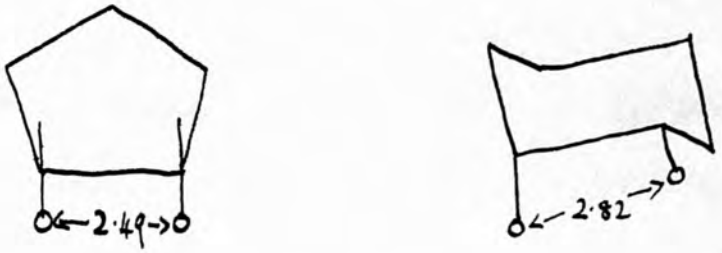
$$H_2[Sn(OH)_6].(bzd.HCl)_2$$

Chelates formed between diphenols, such as catechol or 3,4-dihydroxy-benzaldehyde and inorganic anions, including that from Sn^{IV}, have been studied by ion exchange, electrophoretic, paper chromatographic and spectrophotometric methods.[88] Electrophoretic studies

showed higher mobilities for the chelates, whereas, on paper chromatography, the ionic chelates had lower R_F values than the parent phenols. The ultraviolet spectrum of 3,4-dihydroxy-benzaldehyde was seriously distorted by chelation with stannate ions.

The interaction of germanate and stannate ions with polyols is dependent on the O-O distances in the ions, and the type of diol groupings in the polyols concerned. The O-O distances in these different types of diol grouping vary and can be related to the reactions of polyols with inorganic ions.

The O-O distance in a cis-1,2-diol of a 5-membered planar ring is 2.49 Å. That in a cis-1,2-diol in a 6-membered ring in the chair conformation is 2.82 Å.[89]



The O-O distance in a trans (or threo-)-1,2-diol in an acyclic polyol im the planar-zig-zag arrangement is also 2.82 Å.

81

Lindberg and Swan have measured the mobilities of polyols in germanate solution, and have concluded that the germanate ion complexes with cis-1,2-diols in furanosides and pyranosides, and trans-1,2-diols in acyclic polyols.

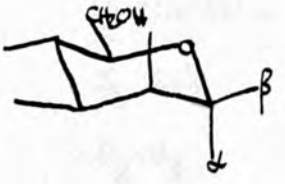
From this information, the M_G values of some of the hexoses, can be correlated with the number of cis-1,2-diol groupings in the chair conformations.

The M_G ($M_{Glucose}$) value of a compound is

$$\frac{\text{The distance moved by the compound or an electrophoretogram}}{\text{The distance moved by glucose in the same time.}}$$

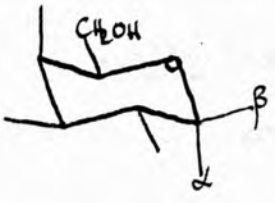
$C_{1\alpha}$-C_2 M_G 1.0

D-Glucose



D-Mannose M_G 1.4

$C_{1\beta}$-C_2, C_2-C_3



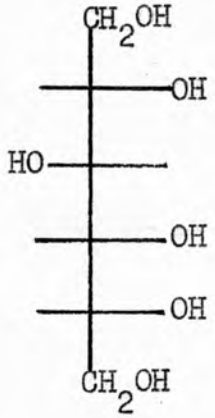
D-Galactose M_G 1.3

$C_{1\alpha}$-C_2, C_3-C_4

D-Allose M_G 1.8

$C_{1\alpha}$-C_2, C_2-C_3, C_3-C_4

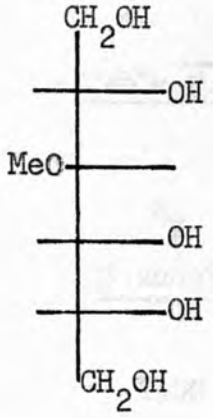
Similarly, when considering the acyclic polyols, the M_G values of the derivatives of D-glucitol can be related to the number of trans-1,2-diol groupings in the molecules.



D-glucitol

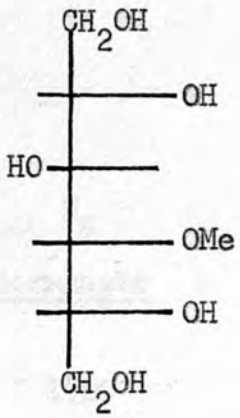
M_G 1.9

C_2-C_3, C_3-C_4



3-O-methyl-D-glucitol

M_G 0.8



4-O-methyl-D-glucitol

M_G 1.4

C_2-C_3

In considering the cyclitols, Lindberg and Swan[81] suggest that the mobilities may be due to complexes with cis-1,2-diols, However, scyllo-inositol, with an M_G value in germanate of 0.2 has no such diol grouping.

There is little sign of the formation of a germanate complex with a cis-1,2-diol grouping in an acyclic polyol (i.e. an erythro-diol grouping)

The results obtained when electrophoresis was carried out using a solution of sodium stannate in water (2%-pH 11.5) are given in Table V[81], in comparison with values for electrophoresis in germanate solution (converted from M_G to M_S ($M_{D\text{-glucitol}}$)).

Table V

Compound	M_S Stannate	M_S Germanate
D-Glucitol	1.00	1.00
D-Mannitol	0.93	1.00
Galactitol	0.99	1.10
D-Arabitol	0.95	0.95
D-Xylitol	1.00	0.89
D-Allitol	0.88	-
D-Altritol	0.95	-
D-Erythritol	0.57	0.53
Glycerol	0.23	0.21
3-O-Methyl-D-Glucitol	0.30	0.42
2-O-Methyl-D-Mannitol	0.88	-
1,2-di-O-methyl-D-Mannitol	0.66	-
2-deoxy-D-Ribitol	0.25	-

Compound	M_S Stannate	M_S Germanate
1-deoxy-D-Altritol	0.80	-
1,6-dideoxy-D-Altritol	0.45	-
1,6-dideoxy-Galactitol	0.72	-
1-deoxy-D-Arabitol	0.58	-
5-deoxy-D-Arabitol	0.78	-
1-deoxy-D-Xylitol	0.88	-
1-deoxy-D-Talitol	0.86	-
1,6-dideoxy-D-Mannitol	0.67	-
1-deoxy-D-Mannitol	0.94	-
Cellobiitol ($\beta 1 \rightarrow 4$)	0.82	0.63
Maltitol ($\alpha 1 \rightarrow 4$)	0.81	-
Lactitol ($\beta 1 \rightarrow 4$)	0.82	0.79
Gentiobiitol ($\alpha 1 \rightarrow 6$)	0.89	-
Sophoritol ($\beta 1 \rightarrow 2$)	0.85	-
D-glucose	0.6-0.75	0.53
D-Mannose	1.00	0.74
D-Galactose	0.78	0.69
D-Gulose	1.07	-
D-Arabinose	0.84	0.79
D-Xylose	0.81	0.73

Compound	M_S Stannate	M_S Germanate
D-Lyxose	1.15	1.00
D-Ribose	1.04	1.10
Sophorose	0.57	-
Maltose	0.52	0.21
Lactose	0.58	0.37
Cellobiose	0.62	0.16
Laminaribose	0.75	0.58
Leucrose	0.82	-
Gentiobiose	0.65	0.53
Isomaltose	0.58	0.47
Sucrose	0.41	0.05
Fructose	0.91	1.10
Sorbose	0.94	1.05
6-deoxy-D-Gulose	1.05	-
2-deoxy-D-Galactose	0.23	-
2-deoxy-D-Allose	0.52	-
3-O-methyl-D-Glusose	0.78	-
5-deoxy-D-glucose	0.63	-
2-deoxy-D-Glucose	0.31	-

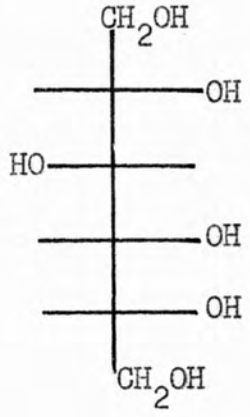
Compound	M_S Stannate	M_S Germanate
3,4-di-O-methyl-D-Mannose	0.71	-
6-deoxy-D-Glucose	0.63	-
6-deoxy-D-Mannose	1.00	-
Methyl α-D-Glucopyranoside	0.28	0
Methyl β-D-Galactopyranoside	0.43	0.26
1,6-Anhydro-2-deoxy-β-D-gulopyranoside	0	-
1,6-Anhydro-β-D-glucopyranoside	0	0
" " " " galactopyranoside	0.81	-
" " " " mannopyranoside	0.96	-
" " " " gulopyranoside	0.77	-
" " " " altropyranoside	0.80	-
Butane-2,3-cis-diol (erythro)	0.019	-
Butane-2,3-trans-diol (threo)	0.04	-
Propane 1,2-diol	0.23	-
Ethane 1,2-diol	0.23	-

Compound	M_S Stannate	M_S Germanate
Myoinositol	0.48	-
Scylloinositol	0.56	-
(+)inositol	0.55	-
Alloinositol	1.00	-
Epiinositol	1.01	-
Mucoinositol	0.67	-
Quebrachitol	0.39	-
l-vibo-quercitol	0.39	-

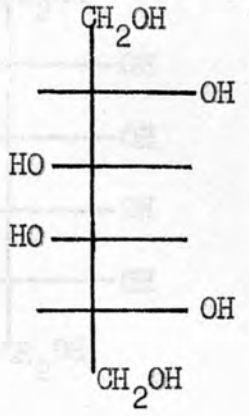
It is interesting to consider the mobilities of the threo- and erythro-butane-2,3-diols. The O-O distance, 2.82 Å in the threo-compound is close to the O-O distance in the stannate ion (2.77 Å) and reaction is expected. The mobility of the erythro-diol is about one half of that of the threo-diol. It is reported that complexing between this group and the O-O groups in the germanate (2.64 Å) is not apparent.[81] It is possible that the O-O distance in the stannate ion is large enough to permit the formation of a non-planar five-membered ring, and that it is this type of complex which is responsible for the migration of cis-butane-2,3-diol.

The order of mobilities of the acyclic polyols, in stannate

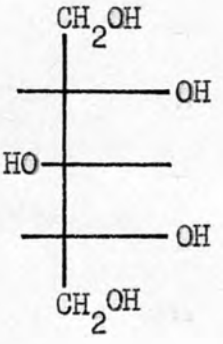
(hexitols and pentitols), is related to the number of threo-diol groupings in each polyol. D-Glucitol (M_S 1.00), galactitol (M_S 0.99) and D-Xylitol (1.00) all contain 2 threo-diol groupings



D-glucitol

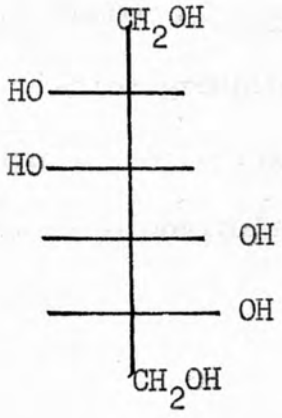


Galactitol

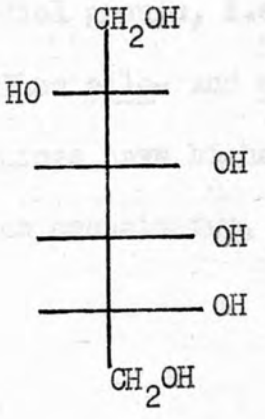


D-Xylitol

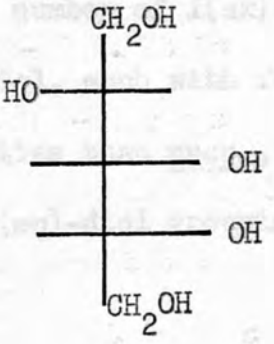
D-Mannitol (0.93), D-Altritol (0.95) and D-Arabitol (0.95) each contain one threo-diol grouping.



D-Mannitol

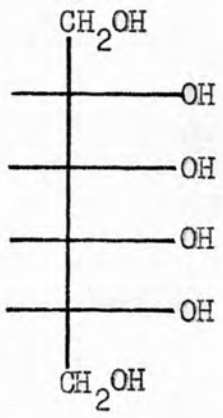


D-Altritol



D-Arabitol

D-Allitol, which has an M_S value of 0.88 - lower than any other hexitol or pentitol studied - has no threo-diol grouping.



D-Allitol

3-O-Methyl-D-Glucitol has no threo-diol groupings and its M_S value is 0.30 compared with the value of 1.00 for D-glucitol.

Of the cyclitols which have been studied (with the exception of scylloinositol) the mobilities are related to the number of cis-1,2-diol groups, i.e. to the number of 1(ax), 2 (eq)-diol groupings. Thus allo- and epi-inositol, each with four 1(ax), 2(eq)-diol groupings have higher mobilities than muco-, myo- and (+)-inositols, which contain two, 1(ax), 2(eq)-diol groupings.

epi-inositol

M_S 1.01

allo -inositol

M_S 1.00

muco-inositol

M_S 0.67

myo-inositol

M_S 0.48

(+)-inositol

M_S 0.55

Quebrachitol (or 1-O-methyl-L-inositol) has an M_S value of 0.39, and contains only one 1 (ax), 2(eq) diol grouping.

Quebrachitol

M_S 0.39

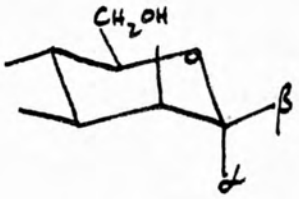
The exception to this reasoning is the M_S value (0.56) for scyllo-inositol, which in its stable conformation has six equational hydroxyl groups, and might therefore be expected to have an M_S value of 0.

scyllo-inositol

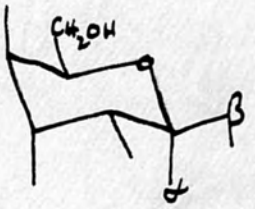
The formation of a tridentate structure, as in some complexes formed between borate ions and polyols[90] may be possible. But in this case two stannate ions per molecule of scyllo-inositol would be necessary.*

In considering the pentoses and hexoses, the M_S values appear to be related to the number of 1 (ax), 2(eq)-diol groupings in the molecule. Thus, D-mannose and D-gulose, have higher mobilities than D-glucose and D-galactose.

* compared with the M_S values for the other inositols an M_S value of 0.56 indicates two complexing sites



D-Mannose



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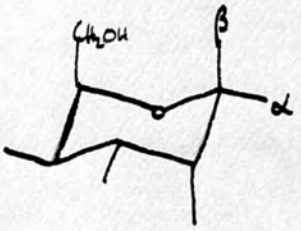
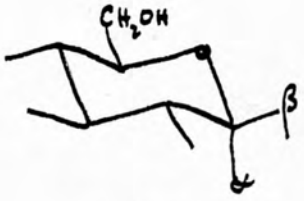
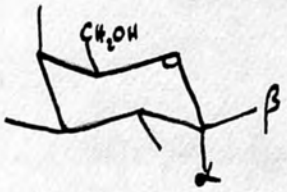


D-Gulose

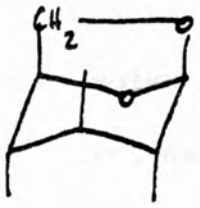


D-Glucose

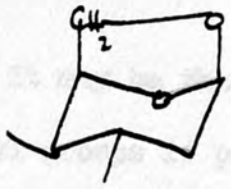


D-Galactose

The M_S values of the anhydro-sugars examined, are in agreement with the theory that 1 (ax), 2(eq)-diol groupings complex with the stannate ion. 1,6-Anhydro-β-D-glucopyranoside and 1,6-anhydro-2-deoxy-β-D-gulopyranoside have M_S values of zero, whereas the other anhydro sugars studied all give evidence of complexing with stannate ions.

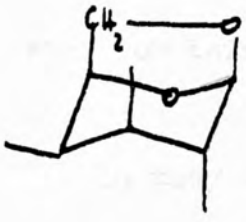


$M_S = 0$

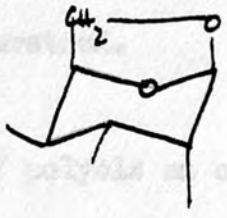


$M_S = 0$

1,6-anhydro-β-D-glucopyranoside 1,6-anhydro-2-deoxy-β-D-gulopyranoside

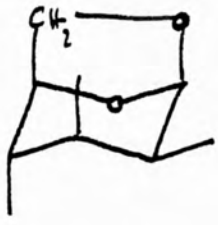


$M_S = 0.81$



$M_S = 0.77$

1,6-anhydro-β-D-galactopyranoside 1,6-anhydro-β-D-gulopyranoside



$M_S = 0.96$

$M_S = 0.80$

1,6-anhydro-β-D-mannopyranoside 1,6-anhydro-β-D-altropyranoside

In contrast to the M_S values of polyols in germanate solution, methyl-α-D-glucopyranoside has an M_S value of 0.28 in stannate solution.

In germanate solution the M_G value is 0. It may be that complexing between adjacent hydroxyl and hydroxymethyl groups is possible, in this case.

As in the case of electrophoresis in germanate solution, the M_S value of 3-O-methyl-D-glucose is higher than that of D-glucose. The reason for this fact is not fully understood.

In many cases, the mobilities of polyols on electrophoresis in stannate solution are similar to values obtained using germanate solution. In considering the merits of these two electrolytes, it can be noted that the sodium stannate used for the electrolyte is very much cheaper than the germanium counterpart.

EXPERIMENTAL

EXPERIMENTAL

Expt. 1 Preparation of D-Mannitol trisphenylboronate

A solution of phenylboronic anhydride, 8.6 g., 1 mol. (≡ 3 mol. phenylboronic acid) in methanol (25 ml.) was added to a solution of D-mannitol, 5 g., 1 mol., in water (25 ml.), with stirring. After 30 min. the white precipitate was filtered off, washed with hot water and cold methanol, and dried. It was recrystallised from dry hexane and had m.p. 137°C. Yield 13 g. (95%); IR showed no -OH groups. The compound had analysis figures, Fd. C 62.9%; H 4.9%; B 7.4%. Calc. for $C_{24}H_{23}O_6B_3$, C, 63.5%; H, 5.2%; B, 7.5%.

Expt. 2 Preparation of D-glucitol trisphenylboronate

A solution of phenylboronic anhydride, 8.6 g., 1 mol. (≡ 3 mol. phenylboronic acid) in methanol (25 ml.) was added to a solution of D-glucitol, 5 g., 1 mol., in water (25 ml.), with stirring. After 30 min. the white precipitate was filtered off, washed with hot water and cold methanol, and dried. It was recrystallised from dry hexane and had m.p. 189°C. Yield 13 g. (95%); IR showed no -OH groups. The compound had analysis figures, Fd. C 62.8%; H 4.8%; B, 7.4%, Calc. for $C_{24}H_{23}O_6B$ C, 63.5%; H, 5.2%; B, 7.5%.

Expt. 3 Attempted preparation of D-mannitol and D-glucitol bis- and monophenylboronates

A solution of phenylboronic anhydride, 2.9 g., 0.66 mol. (≡ 2 mol. phenylboronic acid) in methanol (20 ml.) was added to a solution of D-mannitol, 2.5 g. 1 mol. in water (25 ml.) with stirring. The white precipitate deposited was filtered off and washed and recrystallised as in Expts. 1 and 2. It had m.p. 137°C and IR showed no -OH groups. It was, therefore, D-mannitol trisphenylboronate. When the D-mannitol was replaced by D-glucitol the solid precipitate was D-glucitol trisphenylboronate, m.p. 189°C, and IR showing no -OH groups.

A solution of phenylboronic anhydride, 1.45 g., 0.33 mol. (≡ 1 mol. phenylboronic acid) in methanol (15 mol.) was added to a solution of D-mannitol, 2.5 g. 1 mol. in water (25 ml.) with stirring. The white precipitate had m.p. 137°C and IR showed no -OH groups (D-mannitol trisphenylboronate). When the D-mannitol was replaced by D-glucitol, the precipitated solid had m.p. 189°C, and IR showed no -OH groups (D-glucitol trisphenylboronate).

Expt. 4. Preparation of galactitol bisphenylboronate

A solution of phenylboronic anhydride, 8.6 g., 1 mol. (≡ 3 mol. phenylboronic acid) in methanol (25 ml.) was added to a solution of galactitol, 5 g., 1 mol. in water (150 ml.) with stirring. The mixture was allowed to stand for 1 hr. and the solid precipitate had m.p. 125-135°C, and IR showing -OH absorption.

A solution of phenylboronic anhydride, 5 g., 0.66 mol. (≡ 2 mol. phenylboronic acid) in methanol (25 ml.) was added to a solution of galactitol, 4.4 g. 1 mol. in water (150 ml.). The white precipitate was filtered off, washed with hot water and cold methanol, and dried. It had m.p. 125-130°C and IR absorption at 3250 $cm.^{-1}$. Yield 7 g. (75%), analysis figures, Fd. C, 59.9%; H, 6.0%; B, 6.3%; Calc. for $C_{18}H_{20}O_6B_2$. C, 60.8%; H, 5.6%; B, 6.2%.

Expt. 5. The Preparation of D-Arabitol bisphenylboronate

Using the procedure described in Expt. 1, D-Arabitol, 500 mg. 1 mol. in water (5 ml.) was treated with phenylboronic anhydride, 685 mg., 1 mol. (≡ 3 mol. phenylboronic acid) in methanol (5 ml.). The white precipitate had m.p. 114-116°C, and IR absorption at 3350 $cm.^{-1}$. The yield was 900 mg. (82%), and analysis figures, Fd. B, 6.7%; Calc. for $C_{17}H_{18}O_5B_2$, B, 6.8%.

Expt. 6. The preparation of D-erythritol bisphenylboronate

Using the procedure described in Expt. 1, D-erythritol 1 g. 1 mol. in water (15 ml.) was treated with phenylboronic anhydride 1.7 g. 0.66 mol. (≡ 2 mol. phenylboronic acid) in methanol (15 ml.). The white precipitate had m.p. 88°C and IR showed no -OH absorption. The yield was 2 g. (80%) and analysis figures, B, 7.4%; Calc. for $C_{16}H_{16}O_4B_2$; B, 7.5%.

Expt. 7. Preparation of glycerol monophenylboronate

A solution of phenylboronic anhydride, 2.26 g., 0.33 mol. (≡ 1 mol. phenylboronic acid), in methanol (10 ml.) was added to a solution of glycerol, 2 g. 1 mol. in water (10 ml.) with stirring. There was no precipitate, even when the mixture was allowed to stand overnight.

Glycerol (20 g., 10 mol.) was added slowly to the mixture, with stirring, until the mixture solidified. The white precipitate was filtered off, washed with hot water and cold methanol, and recrystallised from dry hexane. It had m.p. 74.5-76.5°C, and IR showed absorption at 3300 $cm.^{-1}$. The yield was 2.5 g., (65% with respect to phenylboronic acid) and analysis figures, Fd. C, 60.0%; H, 6.1% B, 6.0%. Calc. for $C_9H_{11}O_3B$, C, 60.7%; H, 6.2%; B, 6.2%.

Expt. 8. Preparation of D-glucose bisphenylboronate

A solution of phenylboronic anhydride, 5.8 g., 0.66 mol. (≡ 2 mol. phenylboronic acid) in methanol (20 ml.) was added to a solution of D-glucose, 5 g., 1 mol. in water (20 ml.). The mixture was allowed to stand overnight. An oil separated from the aqueous solution and settled to the bottom of the tube. The oil solidified when it was kept at 5°C for some time, and was recrystallised from benzene/petroleum ether (60-80°C). It had m.p. 166°C and IR showed -OH absorption at 3375 $cm.^{-1}$. The yield was 7 g. (63%) and analysis figures, C, 61.2%; H, 5.3%; B, 6.1%; Calc. for $C_{18}H_{18}O_6B_2$, C, 61.3%; H, 5.1%; B, 6.2%.

Expt. 9. Preparation of 1,3:2,4-di-O-ethylidene-D-glucitol monophenylboronate

1,3:2,4-di-O-ethylidene-D-glucitol, 200 mg. 1 mol. and phenylboronic anhydride, 89 mg., 0.33 mol. (≡ 1 mol. phenylboronic acid) were dissolved in acetone (25 ml.) and refluxed for 1 hr. The acetone was removed by distillation and the solid which was recrystallised from hexane, had m.p. 88°C, and IR showed no -OH group absorption. The yield was 270 mg. (97%) and analysis figures were Fd. C59.6%; H 6.1%; B, 3.3%; Calc. for $C_{16}H_{21}O_6B$, C, 60.0%; H, 6.5%; B, 3.4%.

Expt. 10. Preparation of 2,4-mono-O-benzylidene-D-glucitol-bisphenyl boronate

2,4-mono-O-Benzylidene-D-glucitol, 150 mg., 1 mol., and phenylboronic anhydride, 116 mg., 0.66 mol. (≡ 2 mol. phenylboronic acid) were dissolved in acetone and refluxed for 1 hr. After the removal of the acetone the solid which was recrystallised from hexane, had m.p. 199°C, and IR showed no -OH group absorption. The yield was 240 mg. (98%) and the analysis figures were Fd. C 67.5%; H 5.0%; Calc. for $C_{25}H_{24}O_6B_2$, C 67.8%; H, 5.4%.

Expt. 11. Preparation of 1,6-di-O-benzoyl-D-mannitol-bisphenylboronate

1,6-di-O-Benzoyl-D-mannitol, 100 mg., 1 mol. and phenylboronic anhydride, 55 mg., 0.66 mol. (≡ 2 mol. phenylboronic acid) were dissolved in acetone, and refluxed for 1 hr. After removal of the acetone, the solid, which was recrystallised from hexane, had m.p. 150°C and IR showed no -OH absorption. The yield was 135 mg. (95%) and analysis figures, Fd. C, 67.9%; H, 4.9%; Calc. for $C_{32}H_{28}O_8B_2$, C, 68.3%; H, 5.0%.

Expt. 12. Attempted preparation of a phenylboronate of 1,3:4,6-di-O-methylene-galactitol

1,3:4,6-di-O-methylene-galactitol, 100 mg. 1 mol. and phenylboronic anhydride, 50 mg. 0.33 mol. (≡ 1 mol. phenylboronic acid) were refluxed in acetone and in benzene. In both cases removal of the solvent left a mixture of 1,3:4,6-di-O-methylene-galactitol and phenylboronic anhydride, with m.p. 190-235°C and IR showing -OH absorption.

Expt. 13. Preparation of methyl α-D-glucopyranoside-4,6-phenylboronate

Methyl α-D-glucopyranoside, 3.2 g. 1 mol., and phenylboronic anhydride 1.7 g., 0.33 mol. (≡ 1 mol. phenylboronic acid), were dissolved in benzene (100 ml.) and refluxed, using a Dean and Stark apparatus, until no more water was produced. On evaporation of the benzene, a white solid was deposited. After recrystallisation from benzene it had m.p. 166.5°C, and IR showed reduced -OH absorption. The yield was 4.3 g. (88%) and the analysis figures Fd. B, 3.8%; Calc. for $C_{13}H_{17}O_6B$, B, 3.8%.

Expt. 14. Preparation of 1,6-di-O-benzoyl-2,3,4,5-tetra-O-acetyl-galactitol

Galactitol bisphenylboronate, 1 g. 1 mol. was dissolved in dry pyridine (15 ml.) and redistilled benzoyl chloride, 1 ml. 3 mol. was added to the cold solution. The mixture was allowed to stand overnight at room temperature, and cold water (15 ml.) was then added. Sulphuric acid (1N, 25 ml.) was added and the white precipitated filtered off and dried. This was treated with an acetylating mixture (acetic anhydride/glacial acetic acid/conc. H_2SO_4, 35:15:1) and the mixture allowed to stand overnight. The mixture was then poured on to ice, and the white precipitate produced was filtered, dried and recrystallised from glacial acetic acid. It had m.p. 218-223°C, and IR showed no -OH absorption, and -C=O group absorption at 1700 $cm.^{-1}$ The yield was 150 mg. (< 10%) and analysis figures Fd. C, 59.5%; H, 5.4%; Calc. for $C_{28}H_{30}O_{12}$ C, 60.2%; H, 5.4%).

Expt. 15. Preparation of 2,5-di-O-benzoyl-1,3,4,6-tetra-O-acetyl galactitol

Galactitol bisphenylboronate, 1 g. 1 mol. was dissolved in dry pyridine and redistilled benzoyl chloride, 0.66 ml. 2 mol. was added to the cold solution. The mixture was allowed to stand overnight at room temperature, and the pyridine was then removed

at 1 atmos. pressure, leaving a syrup which was allowed to stand overnight at 5°C. The resulting glue was treated with an acetylating mixture (acetic anhydride/glacial acetic acid/conc. H_2SO_4, 35:15:1) overnight, and the mixture was then poured on to ice. The white solid produced was filtered, and recrystallised from absolute ethanol. It had m.p. 156°C and IR showed no -OH peaks and a -C=O peak at 1700 $cm.^{-1}$ The yield was 150mg. (10%) and analysis figures Fd. C, 59.6%; H, 5.7%; Calc. for $C_{28}H_{30}O_{12}$; C, 60.2%; H, 5.4%.

Expt. 16. The Tosylation of Galactitol bisphenylboronate

Galactitol bisphenylboronate 1 g. 1 mol. was dissolved in dry pyridine (25 ml.) and p-toluene-sulphonyl chloride, 1.6 g. 3 mol., was added to the cold solution. The mixture was allowed to stand at room temperature for 48 hr. Chromatography of the mixture (under conditions causing hydrolysis of the phenylboronate ester groups) in solvent (a), a strong spot with R_F 0.18 (galactitol) and very faint spots with R_F values 0.37 and 0.55, possibly mono- and di-tosyl galactitol. On pouring the mixture on to ice, there was no precipitate.

When a similar tosylation mixture was refluxed at 115°C (the b.p. of pyridine) there was no reaction.

Expt. 17. Methylation of Galactitol bisphenylboronate

Galactitol bisphenylboronate, 500 mg., 1 mol., was dissolved in dry dimethylformamide (10 ml.) and redistilled methyl iodide, 0.155 ml., 2 mol. was added, with excess silver oxide, 0.6 g. The mixture was shaken overnight, and chromatography in solvent (a) showed galactitol (a faint spot) and a series of compounds with R_F values greater than that of galactitol.

Expt. 18. Preparation of galactitol-bisphenylboronate bisurethane and galactitol bisurethane

Galactitol bisphenylboronate, 5 g., 1 mol. was suspended in dry benzene and phenyl isocyanate, 3.5 ml., 2 mol. was added. The mixture was refluxed for 18 hrs. On cooling the solution, a white precipitate was deposited. This was recrystallised from benzene and had m.p. 223-224°C. Yield 1 g. (12%). Analysis figures, Fd. C, 64.7%; H, 5.3%; N, 4.7%; Calc. for $C_{32}H_{30}N_2O_8B_2$, C, 64.8%; H, 5.1%, N, 4.7%.

The galactitol bisphenylboronate was dissolved in dioxan (5ml.) and water (25ml.) was added. The white precipitate formed was filtered, and had m.p. 257°C. Analysis figures, Fd. C,56.8%; H,5.6%; N,6.6%; Calc. for $C_{20}H_{22}N_2O_8$, C,57.0%; H,5.7%; N,6.7%.

Expt.19. Attempted periodate oxidation of galactitol bisurethane

An attempt to prepare a solution of galactitol bisurethane in water was unsuccessful. In addition this compound is not soluble in dioxan/water mixtures, even with more than 50% dioxan in the mixture. Therefore periodate oxidation was impossible.

Expt. 20. Attempted lead tetraacetate oxidation of galactitol bisurethane

Galactitol bisurethane, 174mg., was dissolved in glacial acetic acid, and an excess of lead tetraacetate was added.

The consumption of lead tetraacetate was estimated by first adding a solution of potassium iodide and potassium acetate to the titre, and then estimating the iodine released with sodium thiosulphate solution.

$$Pb(OAc)_4 + 4KI \longrightarrow 4KAc + PbI_2 + I_2$$

It was found that after 2 days the uptake of lead tetraacetate was 5.5 moles per mole of galactitol bisurethane.

Lead tetraacetate oxidations can be carried out in solvents other than glacial acetic acid. Galactitol bisurethane is soluble in dioxan. Lead tetraacetate was added to dioxan and a yellow precipitate was immediately produced.

Expt. 21. Preparation of 1,3:4,6-di-O-benzylidene-galactitol

Using 16g. of galactitol a sample of 1,3:4,6-di-O-benzylidene-galactitol was prepared according to the method of Haskins, Hann and Hudson.[24] 15g.(50%) were obtained, with m.p.215-220°C.

Expt. 22. Preparation of 1,3:4,6-di-O-benzylidene-2,5-bisurethane-galactitol

1,3:4,6-di-O-benzylidene-galactitol,5g.,1mol.,was dissolved in dimethylformamide (50ml.) and phenyl isocynate,3ml., 2mol., was added. The solution was warmed for 30 min. and a white precipitate was produced. This had m.p. 343°C. Yield 5.5g.(65%). Analysis figures, Fd. C,68.2%; H,5.3%; N4.8%; Calc. for $C_{34}H_{32}N_2O_8$, C 68.5%; H,5.4%; N,4.7%.

Expt.23. Preparation of 2,5-bisurethane of galactitol

1,3:4,6-di-O-benzylidene-2,5-bisurethane-galactitol, 5g. was suspended in water (250ml.) and refluxed in the presence of Amberlite resin, IRA 400 (H^+), and a little dioxan. The solution was filtered and the resin washed with dioxan. Water was added to the dioxan solution and the white precipitate was filtered. It had m.p. 257°C, and mixed m.p. with the bisurethane from Expt.18. also 257°C. Analysis figures Fd. C,56.8%; H,5.6%; N,6.6%, Calc, for $C_{20}H_{22}N_2O_8$, C,57.0%; H,5.7%; N,6.7%.

Expt. 24. Tosylation of glycerol monophenylboronate

Glycerol monophenylboronate 1g.,1mol., was dissolved in pyridine (10ml.), and tosyl chloride,1.1g.,1 mol., was added to the cold solution. The mixture was allowed to stand overnight at room temperature. Chromatography in solvent (a) revealed glycerol and no other compounds with R_F values greater than that of glycerol.

Expt. 25. Methylation of glycerol monophenylboronate

Glycerol monophenylboronate,3g.,1 mol., was dissolved in dimethylformamide (50ml.) and methyl iodide 1 ml., 1 mol., was added with silver oxide, 3g. The mixture was shaken overnight, and chromatography in solvent (a) revealed a mixture of glycerol and a

compound with an R_F value greater than that of glycerol, presumably a mono-O-methyl glycerol.

Expt. 26. Preparation of glycerol monophenylboronate monourethane

Glycerol monophenylboronate, 5g., 1 mol., was dissolved in benzene(100 ml.) and phenyl isocynate 3 ml., 1 mol., was added. The mixture was refluxed for 6hr. and the benzene then removed by distillation. The white solid was recrystallised from benzene/petroleum ether 60-80°C. It had m.p.117°C. Yield 5g.(65%). Analysis figures, Fd. C,64.5%; H,5.3%; N,4.9%; Calc. for $C_{16}H_{16}NO_4B$ C,64.5%; H,5.4%; N,4.7%.

Expt. 27. The isolation of glycerol monourethane.

A solution of glycerol monophenylboronate monourethane in benzene/methanol, was placed on several sheets of Whatman No.3 chromatography paper, as streaks. The papers were developed using solvent (a), and the glycerol monourethane was eluted with methanol. The methanolic solution was evaporated to give an oil. On distillation at 0.01 mm.Hg., a mixture of an oil and a crystalline solid (m.p.245°C) was obtained.

Expt. 28. Study of the distillate from Expt. 27.

A concentrated solution of phenylboronic achydride in

methanol was added to a methanolic solution of the distillate from Expt.27. A small quantity of a white precipitate was produced. This had m.p.74.5-76.5°C and IR spectrum identical with that of glycerol monophenylboronate.

Expt. 29. Periodate oxidation of glycerol monophenylboronate monourethane

The periodate oxidations of glycerolmonophenylboronate and glycerol monophenylboronate monourethane, were carried out according to the procedure described in "Organic Reactions" Vol.2. p.341.

A standard solution of sodium arsenite was prepared (0.01N) and using this, an Iodine solution (approx. 0.01N) was standardised. Analar potassium iodide and sodium bicarbonate were used, and the starch solution used as indicator was freshly prepared.

Known weights of phenylboronic anhydride (24.7mg.), glycerol monophenylboronate monourethane (52.3mg.) and glycerol monophenylboronate (44.7mg.),were dissolved in dioxan(25ml.) in volumetric flasks. Water (25ml.) was added , and to each flask, a solution of sodium metaperiodate (10ml.,0.1M) was added. In each case the volume was adjusted to 100 ml. with water.

10 ml. portions of each solution were withdrawn at

intervals, and diluted with 10ml. water. The solutions were neutralised with sodium bicarbonate, 25ml. standard arsenite solution were added, and a little solid potassium iodide dissolved in the solutions. After 15min. the excess arsenite solution was estimated by titration with iodine solution.

The following results were obtained.

Time	mol.IO_4^- uptake by		
	phenylboronic acid	glycerol MPB	glycerol MPB monourethane
0	0	0	0
20min.	0.442	2.27	1.39
50min.	0.442	2.34	1.50
1½hr.	0.523	2.35	1.56
2½hr½	0.547	2.43	1.65
4½hr.	0.590	2.49	1.67
6½hr.	0.630	2.47	1.68
24hr.	0.756	2.68	1.75
28hr.	0.800	2.82	1.81

Expt. 30. Estimation of formaldehyde by chromotropic acid

The chromotropic acid reagent was prepared as follows.[91] Conc.H_2SO_4,(200ml.) was added to water (100ml.). A solution of 0.5g. chromotropic acid was added to 200 ml.of this diluted acid.

2,5-mono-O-methylene-mannitol (8.24mg.) was dissolved in water and excess sodium metaperiodate (0.65g.) was added. The

solution was adjusted to 10 ml. After standing for 1½ hrs. portions of this solution were diluted by different amounts.

1 ml. of each diluted solution was treated as follows:

1 ml. of water was added, followed by 0.1 ml. of 20% Na_2SO_3 solution, and 8.4 ml. chromotropic acid reagent. The solution was heated for 15 min. on a boiling water bath and 0.5 ml. 0.4% thiourea solution was added. (Total volume 10 ml.). The absorption at 5700 Å was read, using a blank containing chromotropic acid.

The following values were obtained:

Conc. of HCHO in mg./litre	absorption at 5700 Å
2.54	0.136
5.08	0.280
9.60	0.530
12.70	0.730

Glycerol monophenylboronate monourethane, 28.0 mg. was dissolved in 25 ml. water. 10 Ml. of this solution was removed, 30 mg. sodium metaperiodate was added and the solution made up to 25 ml. After the solution had been standing for 24 hr., 0.1 ml., and 0.2 ml. portions were removed, diluted to 1 ml., and treated as described with chromotropic acid.

These solutions had absorptions at 5700 Å of 0.276 and 0.590, and indicate respectively that 1.07 and 1.14 moles of HCHO are produced on the periodate oxidation of 1 mol. of glycerol monophenylboronate monourethane.

Expt. 31. Preparation of the formaldehyde derivative of dimedone

To the glycerol monophenylboronate monourethane solution, oxidised with periodate in Expt. 30, a concentrated solution of dimedone in acetone was added. A precipitate was produced, m.p. 190°C (theoretical for formaldehyde derivative of dimedone, is, 189°C). Analysis figures, Fd. C, 69.8%; H, 8.2; Calc. for $C_{17}H_{24}O_4$, C, 70.0%; H, 8.2%.

Expt. 32. Methylation of D-mannitol trisphenylboronate

D-Mannitol trisphenylboronate, 0.22 g. 1 mol. was dissolved in dimethylformamide, and methyl iodide 0.055 ml., 2 mol., was added, with 0.2 g. Ag_2O. The mixture was shaken overnight. Chromatography of the reaction mixture in Solvent (a) showed mainly D-mannitol, on hydrolysis, with a little mono-O-methyl-D-mannitol.

A series of reactions was carried out, in which barium oxide was incorporated into the reaction mixture, in each case in a solution of dimethylformamide.

1. D-Mannitol trisphenylboronate (A) 0.2 g., 1 mol., was treated with methyl iodide, 0.05 ml., 2 mol., in the presence of silver oxide, 0.15 g. and barium oxide, 1 g.
2. A, 0.2 g., 1 mol., was treated with methyl iodide, 0.165 ml., 6 mol., in the presence of silver oxide, 0.2 g. and barium oxide, 1 g.
3. A, 0.2 g.,1 mol., was treated with methyl iodide, 0.5 ml., 15 mol., in the presence of silver oxide, 0.6 g., and barium oxide, 2 g.

4, 5, 6. These reactions mixtures were the same as those in 1, 2 and 3, with the D-mannitol trisphenylboronate replaced by D-mannitol (0.085 g.). In each case the course of the reaction was followed by paper chromatography in solvent (a). The results are enumerated on p. 88

Expt. 33. Attempted partial hydrolysis of D-mannitol trisphenylboronate (A)

1 g. of A, was dissolved in dry dioxan (25 ml.) and the rotation of the solution was observed using a Hilger polarimeter.

Water was added to the solution in 0.03 ml. portions, and after each addition the rotation was measured.

Expt. 34. The reaction of phenyl isocyanate with the compound I

The compound I, 1 g., 1 mol., was dissolved in benzene (50 ml.) and phenyl isocyanate, 0.3 ml., 1 mol. was added. After refluxing for 4 hours, there was no small of phenyl isocyanate in the reaction mixture and chromatography in solvent (a) showed D-glucose in appreciable concentration. Phenyl isocyanate, 5 ml., was added to the mixture and refluxing continued until chromatography in solvent (a) showed that no glucose remained on hydrolysis of the phenylboronate.

The benzene was removed and an oil produced which slowly solidified, to give a white solid, m.p. 47°C. This had analysis figures C, 63.4%; H, 5.9%; and N, 9.2%.

Expt. 35. Tosylation of Compound I and methyl α-D-glucopyranoside-4,6-phenylboronate

Methyl α-D-glucopyranoside-4,6-phenylboronate, 1 g., 1 mol., was dissolved in dry pyridine (15 ml.) and tosyl chloride, 2 g., 3 mol. was added to the cold solution. The solution (i) was allowed to stand at room temperature for 2 days.

The compound I, 1 g., 1 mol., was dissolved in dry pyridine (15 ml.) and tosyl chloride, 2 g., 4 mol., was added to the cold solution. The solution (ii) was allowed to stand at room temperature for 2 days.

Chromatography of the solutions (i) and (ii) in solvent (a) gave the following results:

Solution		Compounds detected
(i)	No methyl α-D-glucopyranoside	Single spot. R_F 0.76 Methyl 2,3-di-O-tosyl-α-D-glucopyranoside
(ii)	Glucose, R_F 0.16	Single spot. R_F 0.69

Expt. 36. The determination of molecular weights

(i) D-Mannitol Trisphenylboronate

Known weights of D-mannitol trisphenylboronate (in the region of 0.03 g.) were added to benzene (10 ml.) and the freezing points of the different solutions were measured in terms of the resistances of the solutions (resistance is a function of temperature).

The weights of D-mannitol trisphenylboronate in given solutions were plotted against R.

The molecular weight was calculated from the formula

$$MW = \text{Gradient} \times K$$

where K is a constant for the apparatus, and M.W. is the molecular weight of the compound.

(ii) and (iii) Glycerol monophenylboronate and glucose bis-phenylboronate

The above procedure was repeated for these two compounds.

Results

(i)		(ii)		(iii)	
Total weight solute in 10 ml.	R	Total weight solute in 10 ml.	R	Total weight solute in 10 ml.	R
0.0353	2871	0.0303	2872	0.0300	2985
0.0695	2876.5	0.0448	2874.5	0.0580	3004
0.0974	2881.5	0.0674	2879	0.0849	3093
0.1417	288.8			0.1141	3154
0.1687	2892.5				

Expt. 37. Chromatography using a non-aqueous solvent

Samples of D-mannitol trisphenylboronate, and D-glucitol tris-phenylboronate in chloroform solution were placed on Whatman No. 1 chromatography paper which had been dipped in a solution of dimethyl sulphoxide in benzene (20%) and dried for a few minutes at 60°C.

The chromatogram was developed with di-iso-propyl ether, saturated

with dimethyl sulphoxide.

The compounds were detected with the periodatocuprate reagent,[92] and had R_F values of 0.

Expt. 38. Ultraviolet spectrum of phenylboronic acid

Phenylboronic anhydride (0.6072 g.) was dissolved in aqueous methanol (50%, v/v; 2 L).

Standard solutions were prepared by diluting 0.5 ml., 1 ml., 2.5 ml., 3 ml., 4 ml. and 5 ml. of this solution to 100 ml. with aqueous methanol (50% v/v), and the absorption measured at 219mμ. The optical density of these solutions was 0.16, 0.29, 0.52, 0.64, 0.81, 1.04, and 1.27.

Expt. 39. Drying of Solvents

(1) Benzene[93]

Cryoscopic benzene was dried by allowing it to stand overnight with $CaCl_2$. It was then decanted and treated with Na wire.

(2) Pyridine[93]

Pyridine was refluxed for 2 hr. over sodium hydroxide pellets. It was then distilled and stored over NaOH pellets.

(3) Dimethylformamide

Dimethylformamide was refluxed over barium hydroxide, and distilled.

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(4) Dioxan

Dioxan was allowed to stand over KOH pellets. It was then distilled and stored in the presence of Na wire.

(5) Hexane

Hexane was stored over solid $CaCl_2$ and filtered quickly when required.

Expt. 40. Infrared Spectroscopy

All spectra were obtained by using the Perkin Elmer, Infracord, Spectrophotometer, 137. The samples used were in the form of a mull with Nujol.

Expt. 41. Paper Chromatography

Paper chromatography was carried out by the descending method, using Whatman No. 1 and Whatman No. 3 paper.

The solvents used were as follows:

(b) n-Butanol-pyridine-water (6:4:3, v/v).

(c) n-Butanol-pyridine-sat. aqueous phenylboronic acid (6:4:2:1 v/v).

(a) n-Butanol-ethanol-water (40:11:19, v/v).

(d) Ethyl acetate-ethanol-water (11:4:2, v/v).

(e) Ethyl acetate-ethanol-sat.aqueous boric acid (11:4:2, v/v).

(f) Ethyl acetate (0.45% phenylboronic acid solution)-ethanol-water (11:4:2, v/v).

(g) Ethyl acetate-acetic acid-water (9:2:2, v/v).

(h) Ethyl acetate (0.55% phenylboronic acid solution)-acetic acid-water (9:2:2, v/v).

Spray reagents for detecting compounds

(1) $AgNO_3/NaOH$ [19]

The paper is dipped in a solution of $AgNO_3$ in acetone, prepared by diluting 5 ml. of sat. aqueous $AgNO_3$ solution and 5 ml. water to 1 litre with acetone.

After allowing the paper to dry in air, it is then sprayed with a solution of sodium hydroxide in ethanol (2%).

Polyols are visible as brown spots on a yellow background.

(2) Potassium periodatocuprate [92]

The paper is sprayed with potassium periodatocuprate solution, when polyols are visible as white spots on a yellow background. The white spots are then sprayed with a solution of rosaniline in acetone.

(3) Potassium permanganate

The paper is dipped in a solution of potassium permanganate in acetone (1%). Diols are visible as yellow spots on a pink

backgrounds but these spots are not permanent.

Expt. 42. Electrophoresis

Electrophoresis was carried out using Whatman No. 3 paper and an electrophoresis machine capable of producing a potential difference of up to 5000 v.

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